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Effect of valproate on renal metabolism in the intact dog

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Effect of valproate on renal metabolism in the intact dog. Valproate is an antiepileptic drug known to induce hyperammonemia in humans. This hyperammonemia might result from a reduced detoxification of ammonium in the liver and/or from an accelerated renal ammoniagenesis. Six dogs with normal acid-base equilibrium and eight dogs with chronic metabolic acidosis were infused with valproate directly into their left renal artery in order to obtain arterial concentrations around 3 to 4 mM. The arterial ammonium concentration rose only in chronically acidotic dogs, whereas the lactate concentration and the lactate/pyruvate ratio increased in both groups. The urinary excretion of lactate and pyruvate increased markedly but the urinary excretion of other relevant metabolites remained minimal. Renal glutamine utilization and ammonium production were not changed by valproate administration in normal dogs but increased modestly in acidotic dogs. However, renal lactate utilization was drastically reduced and in fact, changed into a net production of lactate. Valproate strikingly reduced the renal cortical concentrations of glutamine, glutamate, alphaketoglutarate and citrate, and more modestly those of malate, oxaloacetate, aspartate, alanine and ATP. By contrast, the tissue lactate concentration and the lactate/pyruvate ratio were markedly increased. In experiments with brush border membrane vesicles, valproate inhibited the lactate transporter. These results suggest that high concentrations of valproate drastically inhibited the proximal reabsorption and the proximal and distal oxidation of lactate and pyruvate. Valproate probably became itself a significant energetic substrate for the kidney.

Valproate or dipropylacetate, an eight-carbon branched chain fatty acid, is a widely used antiepileptic drug [1]. Acute [2, 3] and chronic [3–6] hyperammonemia have been reported with its use, possibly due to a reduced rate of ammonium detoxification through urea synthesis in the liver [7, 8], and/or accelerated renal glutamine uptake and ammoniagenesis [9, 10]. The first mechanism was demonstrated in isolated rat liver mitochondria, where valproate inhibited hepatic ureagenesis through depression of mitochondrial synthesis of N-acetylglutamate [11]. The second mechanism is also possibly involved, since intravenous valproate accelerated renal glutamine utilization [10] and increased ammonium release into the renal vein in both man [9, 10] and the rat [12]. Recent in vitro studies have shown that valproate accelerates glutamine utilization and ammoniagenesis in rat [13] and dog [14] renal cortical tubules.

The present study was undertaken to evaluate the potential effects of valproate on renal ammoniagenesis in the intact dog and to determine the sites and mechanisms of the various effects of valproate on renal metabolism. For this purpose, the renal utilization or production and the renal cortical concentrations of relevant metabolites were assessed in dogs with normal acid-base equilibrium and chronic metabolic acidosis before and after valproate administration.

Our data demonstrate that valproate does not change renal glutamine utilization and ammonium production in normal dogs, but modestly increases these parameters in chronically acidotic dogs. Furthermore, valproate strikingly increases the urinary excretion of lactate and pyruvate and markedly inhibits the oxidation of these substrates by the kidney.

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Methods

Fourteen studies were carried out on six normal (18 to 42 kg) and eight chronically acidotic (17 to 23 kg) mongrel dogs. Chronic metabolic acidosis was induced by the daily administration of ammonium chloride (10 g/day) for at least five days. After an overnight fast, each dog was anesthetized with intravenous sodium pentobarbital (30 mg/kg of body weight) and received additional small doses whenever necessary during the experiments. A Harvard respirator was used to ventilate the dogs mechanically through a cuffed endotracheal tube. The rate and depth of respiration were initially adjusted to maintain arterial carbon dioxide tension (PCO_2) around 35 mm Hg. No further attempts were made to prevent arterial PCO_2 variations during the remainder of the experiments.

After a priming dose of creatinine (20 mg/kg of body weight), a 5% mannitol solution containing 2 g of creatinine and 1 g of p-aminohippurate (PAH) per liter was adjusted to pH 7.0, and infused into a jugular vein at a constant rate of 2 ml/min. The abdominal cavity was opened through a midline incision and the left renal vein was catheterized under direct inspection after ligation of the left ovarian or spermatic vein. An isotonic saline solution was infused slowly through the catheter inserted into the left renal vein. During the surgical procedure, each dog received 300 ml of isotonic saline. The left ureter was also catheterized and urine was collected under mineral oil. After careful dissection of the renal pedicle to make sure that only one artery supplied the left kidney, an isotonic saline solution was infused at a rate of 0.25 ml/min through a curved 25-G needle inserted into the left renal artery. If branching of the renal artery was observed, the site of infusion was well before that of branching. During the experiment, blood was drawn

anaerobically through a femoral artery catheter and analyzed immediately for acid-base parameters.

A tissue sample of the right kidney cortex was obtained and instantaneously freeze-clamped between two aluminum blocks cooled to the temperature of liquid nitrogen according to the technique of Wollenberger, Ristau and Schoffa [15]. The interval between removal of a portion of the superficial renal cortex and the freeze-clamping procedure was always less than 5 seconds. The right renal pedicle was ligated immediately after this procedure and the left kidney was studied. Following a 30-minute equilibration period, renal ammoniagenesis and the renal utilization or production of glutamine, glutamate, alpha-ketoglutarate, aspartate, alanine, lactate, pyruvate and citrate by the left kidney were measured by three determinations of arteriovenous differences, and four consecutive, 10-minute urine collections. We have previously shown that unilateral nephrectomy per se does not change renal metabolism and renal metabolite profile 90 minutes after unilateral nephrectomy [16].

Dogs with normal acid-base equilibrium

In the first series of experiments, six dogs were infused directly into their left renal artery for 120 minutes with a half molar solution of sodium valproate, prepared in a normal saline solution by neutralizing valproic acid with sodium hydroxide to pH 7.4. The rate of infusion of valproate was increased from 1 mg/kg/min during the first 30 minutes to 2 mg/kg/min during the second 30 minutes, to 4 mg/kg/min during the third 30 minutes, and finally to 8 mg/kg/min during the last 30 minutes of infusion (a total cumulative dosage of 450 mg/kg). During the infusion of valproate, twelve consecutive, 10-minute urine collections bracketed by nine determinations of arteriovenous differences were obtained. As soon as the last blood sample had been drawn, that is, after 120 minutes of valproate administration, a tissue sample from the remaining left kidney was freeze-clamped as described above and the animal was sacrificed. The renal cortical metabolites were thus determined before and after 120 minutes of valproate administration.

Dogs with chronic metabolic acidosis

In the second series of experiments, eight dogs were infused with the same solution of sodium valproate at a dose of 4 mg/kg/min directly into their left renal artery for 70 minutes. After a 10-minute equilibration period, six consecutive, 10-minute urine collections bracketed by four determinations of arteriovenous differences were obtained. After 70 minutes of valproate administration, a tissue sample from the remaining left kidney was freeze-clamped.

Transport studies

In order to elucidate the origin of the lactaturia induced by valproate, brush border membrane vesicles were prepared from dog kidney cortex by calcium precipitation according to the technique of Evers et al [17]. These vesicles were used to study the effect of valproate on L-lactate transport. The uptake of ^{14}C[U]-L-lactate (40 μM) by freshly prepared vesicles suspended in 50 mM Tris-HEPES buffer was studied under condition of sodium (150 mM out, 0 in) and H^+ (pH 6 out, pH 8 in) gradients in absence or in presence of valproate (0, 0.1, 1 and 5 mM), acetate (5 mM) or 5-hydroxy-cyano-cinnamate (5 mM). Two types of experiments were performed: in the first type, the vesicles were exposed to ^{14}C-lactate for 15, 30, 60, 120, 300, 600 and 3600 seconds, filtered, and were washed with 8 ml sodium-free buffer (pH 6). In the second type of experiments, the transport was studied at a fixed time (30 sec) while the concentration of valproate, acetate, and cinnamate was varied from 0.1 to 10 mM. At the end of the incubation, the vesicles were diluted in 1 ml of an ice-cold lactate-free, sodium-free and pH 6.0 solution, and rapidly filtered on 0.45 μ mesh Amicon filters (Amicon Corp., Lexington, Massachusetts, USA). The filters were then washed with 8 ml of the same solution, dissolved in Filter Count™ (Packard Instruments, Downers Grove, Illinois, USA) scintillation cocktail overnight and counted.

Analytical methods

The pH of blood and urine and the arterial and renal venous blood PO_2 were measured anaerobically at 38°C with a digital acid-base analyzer (Radiometer, model PHM 72, Copenhagen, Denmark). Analytical methods used to determine carbon dioxide content, sodium, potassium, chloride, phosphate, creatinine, and PAH concentrations have been described previously [18, 19]. The plasma valproate concentration was measured by the homogeneous enzyme immunoassay technique of Elyas et al [20]. The frozen tissues were pulverized and extracted with four volumes 10% of wt/vol perchloric acid per gram of tissue. Concentration of metabolites was measured on a neutralized extract of deproteinized blood, urine or tissue by methods previously described [16].

Calculations

Plasma carbon dioxide tension and bicarbonate concentration were calculated from the Henderson-Hasselbalch equation using a solubility coefficient of 0.0301 and a pK′ of 6.10 for carbonic acid. For urine, a solubility coefficient of 0.0309 was used while the pK′ was $6.33 - 0.5 \sqrt{(Na^+) + (K^+)}$, the concentrations of Na and K being expressed in equivalents per liter [21]. Blood oxygen content was calculated from the PO_2, pH, pCO_2, body temperature, hemoglobin and hematocrit as described by Kelman [22]. Exogenous creatinine clearance was used to estimate glomerular filtration rate (GFR). Renal blood flow was calculated from the PAH clearance (corrected for renal extraction) and the hematocrit value. Renal utilization or production of each metabolite took into account the net urinary losses according to the Wolf's equation as modified by Cohen [23] for substances utilized or produced by the kidney. Renal cortical oxaloacetate concentration was calculated by two independent methods based on the assumption that the lactate dehydrogenase, malate dehydrogenase, and aspartate aminotransferase systems are in near equilibrium in the dog kidney cortex [24]. Renal cortical ammonium concentration was calculated from the renal vein ammonium concentration [25]. In order to express the renal utilization or production of metabolites at comparable rates of sodium filtration and reabsorption, these parameters were also expressed per 100 ml GFR. The rationale of this calculation was fully explained in a recent publication [26].

Statistical analysis

Unless otherwise specified, the term significant is used throughout the paper to describe a difference with a P value of less than 0.05. A two-way variance analysis for repeated

Table 1. Effect of progressive infusion of valproate in dogs with normal acid-base equilibrium

	Control	Valproate 1 mg/kg/min	Valproate 2 mg/kg/min	Valproate 4 mg/kg/min	Valproate 8 mg/kg/min
Arterial blood					
Valproate *mM*	0	0.60 ± 0.05[a]	1.38 ± 0.07[a]	3.04 ± 0.10[a]	6.28 ± 0.28[a]
Ammonium *mM*	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.02
Glutamine (plasma) *mM*	0.44 ± 0.04	0.34 ± 0.02[a]	0.32 ± 0.02[a]	0.31 ± 0.03[a]	0.31 ± 0.03[a]
Lactate *mM*	1.50 ± 0.24	1.74 ± 0.33	2.05 ± 0.41	2.34 ± 0.44[a]	2.74 ± 0.50[a]
Pyruvate *mM*	0.09 ± 0.01	0.08 ± 0.004	0.06 ± 0.01	0.05 ± 0.01[a]	0.04 ± 0.01[a]
Lactate/pyruvate	17	22	34	47	69
Urine					
pH	7.04 ± 0.21	7.20 ± 0.11	7.18 ± 0.12	7.25 ± 0.11	7.36 ± 0.07
C_{HCO_3}/GFR %	2.4 ± 0.5	4.3 ± 0.6	4.3 ± 0.5	7.0 ± 1.0[a]	13.4 ± 1.3[a]
C lactate/GFR %	0.7 ± 0.2	3.8 ± 0.8	18.6 ± 1.5[a]	42.7 ± 5.7[a]	81.5 ± 6.9[a]
C pyruvate/GFR %	1.6 ± 0.5	4.9 ± 0.7	19.3 ± 3.0	61.8 ± 11.0[a]	152.1 ± 17.2[a]
GFR *ml/min*	34.4 ± 8.0	32.2 ± 7.8	33.3 ± 7.8	29.2 ± 6.7	22.9 ± 5.6[a]
Renal blood flow *ml/min*	253 ± 25	202 ± 29	185 ± 43	185 ± 58	165 ± 62
Renal utilization or production of metabolites					
Total ammonia production	87 ± 20	87 ± 14	83 ± 8	87 ± 8	99 ± 11
Glutamine utilization	−68 ± 20	−72 ± 16	−60 ± 7	−65 ± 6	−69 ± 11
Lactate utilization	−204 ± 58	−106 ± 38[a]	−27 ± 14[a]	−21 ± 31[a]	18 ± 44[a]
Pyruvate production	19 ± 13	8 ± 8	13 ± 5	14 ± 3	15 ± 5
Citrate utilization	−18 ± 5	−15 ± 4	−15 ± 4	−10 ± 2	−11 ± 2

Values are means ± SE ($N = 6$ dogs) and were calculated from the means of three (during the control period) and two (during the experimental periods) arterial blood values, from the means of four (during the control period) and three (during the experimental periods) 10-min urinary collections or renal utilizations or productions of metabolites in micromoles per 100 ml GFR.

[a] Significant difference from the mean control value, $P < 0.05$

measurements on one level was performed, followed by a comparison of all mean values obtained by the Newman-Keuls procedure [27]. When specified, some comparisons were also made using Student's *t*-test for paired or unpaired data.

Results

Dose-response study of valproate in dogs with normal acid-base equilibrium

The effects of the infusion of progressive quantities of valproate in dogs with normal acid-base equilibrium are depicted in Table 1. Despite the progressive rise in the arterial plasma valproate concentration to values exceeding 6 mM, the arterial ammonium concentration was not significantly increased nor were there significant changes in blood acid-base parameters and plasma electrolytes. Valproate decreased the arterial plasma concentrations of glutamine and citrate, but did not significantly alter the concentrations of glutamate, alphaketoglutarate, aspartate and alanine. A stepwise increment in lactate concentration (reflecting either a decreased lactate utilization or an accelerated production) was observed in parallel with valproate infusion. Because lactate increased in the presence of a decreased pyruvate concentration, the arterial lactate/pyruvate ratio rose progressively and markedly, reflecting a NAD/NADH change in undefined organs.

Valproate administration did not induce a significant bicarbonaturia since the fractional bicarbonate excretion did not exceed 13% with the highest dose of valproate. This can be accounted for in part by the modest volume expansion caused by the infusion of isotonic saline into the left renal vein. This expansion could also account, at least in part, for the modest rise in urine flow and the fractional excretion of sodium, potassium and chloride observed during valproate infusion.

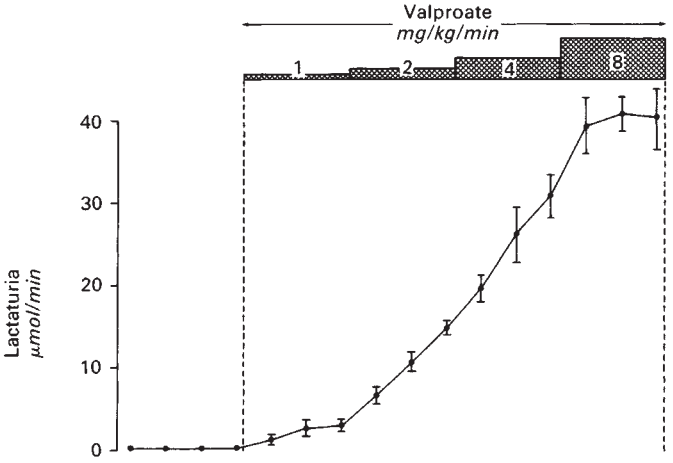

Fig. 1. *Urinary lactate excretion observed before and during the infusion of progressive quantities of valproate in dogs with normal acid-base equilibrium.*

Fractional phosphate excretion remained around 20% before and during valproate infusion. The urinary fractional excretion of glutamine, glutamate, alphaketoglutarate and citrate remained minimal before and during valproate infusion. However, a marked rise in the urinary excretion of lactate (Fig. 1) and pyruvate (the amount excreted into the urine exceeding the filtered pyruvate with the highest dose of valproate) indicates an inhibition of the reabsorption of these organic anions, presumably in the proximal tubule. At plasma valproate concentrations not exceeding 3 mM, no significant renal hemodynamic effects were observed.

Valproate infusion did not significantly modify the production

Table 2. Effect of valproate on arterial blood parameters

	Normal dogs		Acidotic dogs	
	control	valproate	control	valproate
Valproate *mM*	0	3.04 ± 0.10[a]	0	4.66 ± 0.64[a]
pH	7.38 ± 0.01	7.39 ± 0.02	7.08 ± 0.07[b]	7.08 ± 0.04
PCO_2 *mm Hg*	32.2 ± 3.0	34.2 ± 2.8	35.0 ± 0.5	34.3 ± 0.8
Bicarbonate *mM*	18.2 ± 1.5	19.9 ± 1.7	10.8 ± 1.1[b]	10.5 ± 1.0
PO_2, *mm Hg*	NM	NM	105.0 ± 1.9	119.2 ± 4.3
Sodium *mM*	143.9 ± 2.5	147.7 ± 2.5	143.7 ± 0.4	147.1 ± 2.5[a]
Potassium *mM*	3.4 ± 0.1	3.6 ± 0.3	2.8 ± 0.03[b]	3.1 ± 0.4[a]
Chloride *mM*	106.3 ± 1.9	109.1 ± 1.6	124.9 ± 1.1[b]	124.6 ± 1.2
Ammonium *mM*	0.07 ± 0.01	0.08 ± 0.01	0.11 ± 0.01[b]	0.16 ± 0.01[a]
Glutamine (plasma) *mM*	0.44 ± 0.04	0.31 ± 0.03[a]	0.46 ± 0.05	0.43 ± 0.05
Glutamate (plasma) *mM*	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.004	0.02 ± 0.003
Alanine *mM*	0.24 ± 0.02	0.20 ± 0.02	0.55 ± 0.06[b]	0.43 ± 0.04
Lactate *mM*	1.50 ± 0.24	2.34 ± 0.44[a]	1.08 ± 0.20	2.34 ± 0.26[a]
Pyruvate *mM*	0.09 ± 0.01	0.05 ± 0.01[a]	0.10 ± 0.01[b]	0.04 ± 0.01[a]
Lactate/pyruvate	17	47	11	59
Citrate *mM*	0.11 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.05 ± 0.01

Values are means ± SE ($N = 6$ normal dogs and 8 acidotic dogs) and were calculated from the means of three (during the control period) and two (during the experimental periods) blood values. The values presented during valproate infusion were observed when a dose of 4 mg/kg/min was infused in both groups of dogs. NM, not measured.

[a] Significant difference ($P < 0.05$) from the mean control value, the comparisons being made with the Student's *t*-test for paired data.

[b] Significant difference ($P < 0.05$) between the control values in normal and acidotic dogs, the comparisons being made with the Student's *t*-test for unpaired data.

of ammonium nor the utilization of glutamine by the kidney, as both metabolic events occur mostly in the proximal tubules of normal dogs. The ratio of ammonium production to glutamine utilization remained around 1.4, and valproate did not alter the distribution of the ammonium produced between the urinary compartment and the renal venous blood. The renal production of glutamate and alanine did not change significantly and that of alphaketoglutarate and aspartate remained minimal. By contrast, valproate markedly decreased lactate utilization in both the proximal and distal tubules of the dogs with normal acid-base equilibrium. This dose-dependent reduction in lactate utilization was not accompanied by a decreased reabsorption of sodium and chloride. This suggests that a new substrate was now metabolized in the proximal tubules as well as in the more distal thick ascending limb of Henle to support the cellular work, despite the absence of lactate oxidation.

Comparative effects of valproate in normal and chronically acidotic dogs

Arterial blood parameters. In chronically acidotic dogs, the ATP turnover is mainly supported by glutamine in the proximal tubule and by lactate in the distal nephron [28, 29], thus allowing a clear distinction between proximal and distal metabolic effects. For this reason, we decided to compare the renal effects of 3 to 4 mM arterial valproate in normal and acidotic dogs (Table 2). In the control periods, all the effects that were expected with ammonium chloride acidosis were observed in the acidotic dogs; that is lower arterial pH, lower plasma bicarbonate and potassium concentrations, and a higher plasma chloride concentration. Valproate administration had comparable effects in both normal and acidotic dogs on the arterial concentration of lactate and pyruvate and on the arterial lactate/pyruvate ratio. A significant valproate-induced rise in arterial ammonium concentration occurred only in chronically acidotic animals, suggesting that, in the dog, valproate produces systemic hyperammonemia only when a greater load of ammonium, due to an accelerated release into the renal vein, must be detoxified by the liver.

Urinary parameters. A modest rise in urine flow and fractional excretion of sodium and chloride was observed in both groups of dogs during valproate infusion (Table 3). The urinary excretion of lactate and pyruvate increased markedly in all instances. A 3 to 5 mM valproate concentration had no significant effects on renal hemodynamics in the normal dogs, but decreased the glomerular filtration rate and renal blood flow in chronically acidotic dogs.

Renal utilization or production of metabolites. As expected, chronic metabolic acidosis increased renal ammonium production (Table 4). Valproate infusion did not change the release of ammonium into the renal vein nor alter ammonium urinary excretion in normal dogs; however, it increased the urinary excretion of ammonium in the chronically acidotic dogs significantly. The total ammonium production and the renal utilization of glutamine increased modestly in acidotic dogs. The rise in urinary ammonium excretion could not be accounted for by changes in urine flow or pH, but possibly could result from the presence of increased amounts of poorly reabsorbable anions (lactate, pyruvate, valproate, metabolites of valproate) in animals with a distal sodium-hydrogen exchange accelerated by chronic metabolic acidosis.

The renal utilization or production of critical metabolites is presented in Table 4 and the apparent metabolic fluxes are described in Figure 2. In the latter figure, the apparent metabolic fluxes in the kidney are calculated as previously described. The mass conversion through major metabolic pathways or release of metabolites can be estimated. This procedure has the advantage of providing an indirect estimation of the maximal amount of oxidation of the major metabolites extracted by the kidney and has been fully described in recent

Table 3. Effect of valproate on urinary parameters

	Normal dogs		Acidotic dogs	
	control	valproate	control	valproate
Urine flow *ml/min*	1.5 ± 0.4	2.7 ± 0.7[a]	1.8 ± 0.3	2.0 ± 0.3
Urine pH	7.04 ± 0.21	7.25 ± 0.11	6.02 ± 0.71	6.32 ± 0.20
Urine PCO_2 *mm Hg*	61.8 ± 9.1	52.9 ± 8.5	31.4 ± 4.8[b]	39.3 ± 7.0
C_{HCO_3}/GFR %	2.4 ± 0.5	7.0 ± 1.0[a]	1.3 ± 0.2[b]	6.0 ± 2.9
C_{Na}/GFR %	1.0 ± 0.3	3.7 ± 0.7[a]	3.1 ± 0.7[b]	5.2 ± 1.9
C_K/GFR %	23.7 ± 4.7	30.3 ± 3.5	21.1 ± 3.3	20.2 ± 4.6
C_{Cl}/GFR %	0.8 ± 0.2	2.5 ± 0.7[a]	3.6 ± 1.0[b]	4.3 ± 1.5
C lactate/GFR %	0.7 ± 0.2	42.7 ± 5.7[a]	4.7 ± 1.5[b]	30.5 ± 9.6
C pyruvate/GFR %	1.6 ± 0.5	61.8 ± 11.0[a]	4.5 ± 2.1	96.1 ± 17.8
GFR *ml/min*	34.4 ± 8.0	29.2 ± 6.7	21.9 ± 3.4	14.4 ± 2.4[a]
Renal blood flow *ml/min*	253 ± 25	185 ± 58	157.5 ± 37.4	82.0 ± 12.3

Values are means ± SE ($N = 6$ normal dogs and 8 acidotic dogs) and were calculated from the means of four (during the control period) and three (during the experimental periods) 10-min urinary collections. The values presented during valproate infusion were observed when a dose of 4 mg/kg/min was infused in both groups of dogs.

[a] Significant difference ($P < 0.05$) from the mean control value, the comparisons being made with the Student's *t*-test for paired data.

[b] Significant difference ($P < 0.05$) between the control values in normal and acidotic dogs, the comparisons being made with the Student's *t*-test for unpaired data.

Table 4. Effect of valproate on renal utilization or production of metabolites

	Normal dogs		Acidotic dogs	
	control	valproate	control	valproate
$U_{NH_4}V$	24 ± 7	31 ± 7	74 ± 8[b]	110 ± 12[a]
Renal vein ammonium release	64 ± 16	56 ± 12	84 ± 21	77 ± 29
Total ammonium production	87 ± 20	87 ± 8	157 ± 22[b]	187 ± 35
Glutamine utilization	−68 ± 20	−65 ± 6	−73 ± 16	−108 ± 22
Glutamate production	9 ± 3	4 ± 1	9 ± 4	2 ± 5
Alanine production	66 ± 12	53 ± 5	35 ± 14	36 ± 9
Lactate utilization/production	−204 ± 58	−21 ± 31[a]	−134 ± 52	+17 ± 41[a]
Pyruvate production	19 ± 13	14 ± 3	12 ± 10	36 ± 15[a]
Citrate utilization	−18 ± 5	−10 ± 2	−12 ± 5	−14 ± 7

Values are means ± SE ($N = 6$ normal dogs and 8 acidotic dogs) in micromoles per 100 ml GFR and were calculated from the means of four (during the control period) and three (during the experimental periods) values. The values presented during valproate infusion were observed when a dose of 4 mg/kg/min was infused in both groups of dogs.

[a] Significant difference ($P < 0.05$) from the mean control value, the comparisons being made with the Student's *t*-test for paired data.

[b] Significant difference ($P < 0.05$) between the control values in normal and acidotic dogs, the comparisons being made with the Student's *t*-test for unpaired data.

publications [26, 29, 30]. No significant changes in the renal utilization or production of glutamate, alphaketoglutarate, aspartate, alanine and citrate were induced by valproate in normal dogs. In these dogs, the proximal glutamine extraction was entirely accounted for by alanine and glutamate production. No net flux through glutamate dehydrogenase (GLDH) was demonstrated. At least 19 micromoles per 100 ml GFR of unmeasured amino acids were required to explain the mass balance equation between measured amino acid utilization and the production of ammonium (Table 4). A maximum conversion of 203 micromoles per 100 ml GFR of lactate and citrate into CO_2 and glucose was demonstrated.

Valproate did not significantly change the extraction of unidentified amino acids and the production of glutamate and alanine, but a small net deaminating GLDH flux became apparent. The renal lactate utilization was markedly reduced by valproate and even changed into a net lactate production at the highest dose of valproate (Fig. 3). The striking effect of these changes was a marked fall in the apparent oxidation of carbon skeletons of glutamine, lactate and citrate from 203 to 25 micromoles per 100 ml GFR. The maintenance of an unaffected overall kidney function suggests that a new substrate now supported the renal ATP turnover. Thus, this analysis demonstrates a small but significant effect of valproate on proximal tubule metabolism, and a major displacement of lactate oxidation both in the proximal and distal nephron.

Even if the measured renal extraction of glutamine was not significantly different in control and acidotic dogs, several striking changes were induced by acidosis. A significant rise in total ammonium production indicated an accelerated deamidation/deamination of glutamine in the kidney, as reflected by the significant increment in the deaminating GLDH flux. As previously described [29], other non-glutamine-dependent pathways (55 micromoles per 100 ml GFR) contributed significantly to the ammoniagenesis. An apparent oxidation of 163 micromoles per 100 ml GFR of carbon skeletons of glutamine, lactate and citrate was observed.

When valproate was infused into acidotic dogs, the GLDH flux rose markedly, whereas the utilization of unmeasured amino acids decreased. Most of the carbon skeletons of

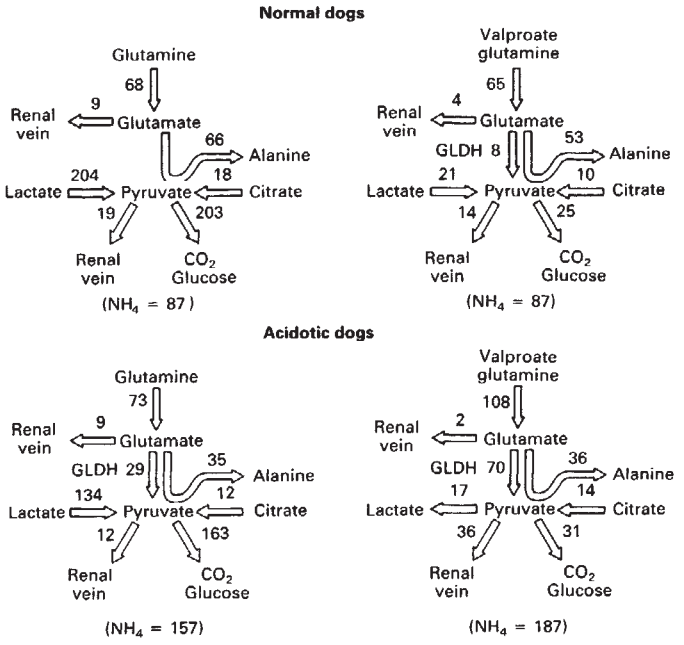

Fig. 2. *Renal metabolism of glutamine and related metabolites in dogs with normal acid-base equilibrium (upper panel) and with chronic metabolic acidosis (lower panel).* Means for glutamine, lactate and citrate extracted and alanine and glutamate produced are given in Table 4 and are expressed as micromoles per 100 ml GFR. Numbers close to each arrow represent the mean flux calculated from the measured values for the reaction(s) represented by the arrow. The various metabolites are shown to join the pyruvate (oxaloacetate) pool supporting both oxidation and gluconeogenesis. The total ammonium production measured is indicated between parentheses. The presentation of the data is fully described in references 29 and 30.

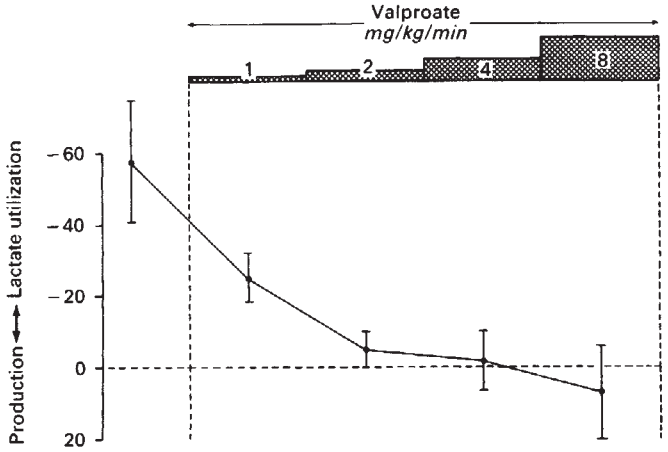

Fig. 3. *Renal utilization of lactate observed before and during the infusion of progressive quantities of valproate in dogs with normal acid-base equilibrium.* The renal utilization of lactate was changed into a net production at the highest dosage of valproate.

glutamine were now released from the kidney as alanine, lactate and pyruvate. It must be pointed out that the renal lactate utilization was now abolished and converted into a net lactate production. The small amount of carbon skeletons now oxidized was comparable to that observed in control dogs infused with valproate. Again, a new source of NADH must support the renal ATP turnover following valproate infusion. Thus the overall effect of valproate in both control and acidotic dogs was to increase the glutamine utilization through the GLDH pathway (proximal effect) and to displace the majority of lactate oxidation (mainly distal effect).

Metabolites profile of the renal cortex

In both normal and acidotic dogs, valproate strikingly reduced the renal cortical concentrations of glutamine, glutamate, alphaketoglutarate, citrate and, more modestly, those of malate, oxaloacetate, aspartate and alanine (Table 5). Furthermore, because the lactate concentration was markedly increased and that of pyruvate only modestly, a four-fold rise in the lactate/pyruvate ratio (decreased cytosolic NAD/NADH ratio) was observed following valproate infusion. The renal cortical lactate concentration (6 mM), which was well above the arterial concentration (2 to 3 mM) despite the markedly decreased proximal reabsorption of lactate, indicated an increased production and/or a reduced utilization of lactate and pyruvate in the kidney cortex. The significant fall in glutamine, glutamate and alphaketoglutarate probably reflected their accelerated transformation into lactate, as suggested on the metabolic flow chart presented in Figure 2. In normal dogs, valproate infusion also increased modestly the concentrations of phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate, whereas it reduced that of glucose-6-phosphate. The concentrations of acetoacetate, β-hydroxybutyrate and fumarate remained minimal and unchanged. Valproate decreased the renal ATP concentration significantly only in dogs with normal acid-base equilibrium and increased inorganic phosphate.

The increased lactate/pyruvate ratio observed in the blood and the renal cortex of normal and acidotic dogs, in relation to the amount of valproate infused, suggested a production of NADH from valproate oxidation, which supported the renal ATP turnover. It should be noted that these metabolite profiles in the renal cortex mainly represented changes in proximal tubules, a nephron segment which predominates in the superficial tissue sample obtained for these measurements. For practical reasons, it was impossible to obtain reliable freeze-clamped tissue representative of the thick ascending limb (red outer stripe of the inner medulla).

Transport studies

Figure 4A presents the uptake of lactate by brush border membrane vesicles as a function of time. It can be seen that a clear overshoot above the equilibrium situation (3600 seconds) is observed after 90 to 120 seconds when Na^+ and H^+ gradients (inside < outside) is established, but not when these gradients are absent. The overshoot is progressively suppressed by increasing concentrations of valproate (from 0.1 to 5 mM). In contrast, 5 mM acetate only modestly affects the lactate accumulation process (not shown). When lactate uptake is examined as a function of the valproate concentration (Fig. 4B), an apparent K_i for valproate around 0.1 mM is found, acetate and cinnamate having little effect on lactate transport and accumulation. We would conclude that these experiments demonstrate a direct inhibitory effect of valproate on a sodium- (and proton-) lactate contransport in dog brush border membrane.

Table 5. Effect of valproate on metabolites profile of the renal cortex

	Normal dogs		Acidotic dogs	
	control	valproate	control	valproate
Glutamine	0.71 ± 0.04	0.22 ± 0.03[a]	0.40 ± 0.06[b]	0.18 ± 0.03[a]
Glutamate	5.09 ± 0.20	2.17 ± 0.22[a]	2.30 ± 0.17[b]	1.05 ± 0.08[a]
α-Ketoglutarate	0.064 ± 0.005	0.025 ± 0.004[a]	0.065 ± 0.014	0.028 ± 0.006[a]
Malate	0.28 ± 0.05	0.18 ± 0.04	0.35 ± 0.11	0.30 ± 0.12
Oxaloacetate (calculated)				
MDH-LDH	5.39	0.86	10.69	2.81
GOT	2.66	1.38	8.94	4.34
Aspartate	1.42 ± 0.22	0.80 ± 0.06	2.12 ± 0.35	1.09 ± 0.23[a]
Citrate	0.14 ± 0.01	0.06 ± 0.01[a]	0.59 ± 0.28	0.33 ± 0.18[a]
Lactate	0.52 ± 0.06	3.66 ± 0.89[a]	0.82 ± 0.20	2.94 ± 0.33[a]
Pyruvate	0.04 ± 0.004	0.07 ± 0.01[a]	0.10 ± 0.02[b]	0.11 ± 0.02
Lactate/pyruvate	13	52	8	27
Alanine	1.05 ± 0.02	0.94 ± 0.09	1.16 ± 0.14	0.70 ± 0.04[a]
ATP	1.73 ± 0.07	1.01 ± 0.06[a]	1.56 ± 0.12	1.50 ± 0.15
ADP	0.58 ± 0.05	0.55 ± 0.06	NM	NM
AMP	0.12 ± 0.02	0.16 ± 0.02	NM	NM
Inorganic phosphate	2.35 ± 0.06	3.59 ± 0.10[a]	2.98 ± 0.41	3.51 ± 0.32
Calculated values				
NAD^+/NADH (LDH)	693	172	1099	337
NAD^+/NADH (GLDH)	1.1	1.3	3.7	4.4

Values are means ± SE ($N = 6$ normal dogs and 8 acidotic dogs) before and at the end of valproate infusion. Results are expressed in micromoles per gram wet weight except for oxaloacetate, expressed in nanomoles. Approximative values in mM can be estimated by multiplying these numbers by two. Abbreviations are: MDH, malate dehydrogenase; LDH, lactate dehydrogenase; GLDH, glutamate dehydrogenase; NM, not measured.

[a] Significant difference ($P < 0.05$) from the mean control value, the comparisons being made with the Student's t-test for paired data.

[b] Significant difference ($P < 0.05$) between the control values in normal and acidotic dogs, the comparisons being made with the Student's t-test for unpaired data.

Discussion

Effects of valproate on renal tubular transport of lactate and pyruvate

Valproate administration markedly reduced the renal reabsorption of lactate and pyruvate in a dose-dependent fashion. This was not due to the observed increased concentration of these substrates in the blood or in the renal cortex. Indeed, the increment in lactate concentration in both tissues was modest, while that of pyruvate remained virtually unchanged. Furthermore, a leak of lactate from the blood back to the lumen of the nephron seems unlikely; usually the urinary lactate excretion does not increase significantly when arterial lactate is increased [31]. This lactaturia was not related to changes in blood or tubular fluid pH, since the same effect was observed in normal and chronically acidotic dogs.

In specific experiments with brush border membrane vesicles, we have demonstrated that valproate exerts a direct and specific effect on the transporter for lactate present in this membrane. This effect occurs at concentrations of valproate below the plasma concentration (around 0.5 mM) induced by oral valproate therapy in man [32]. This luminal transporter is known to be competitively inhibited by different short-chain fatty acids [33] and, as shown here, by valproate itself. A specific inhibition of lactate (and pyruvate) reabsorption by proximal tubules is therefore the most likely mechanism of the observed lactaturia.

The absence of a significant effect on sodium, bicarbonate and phosphate reabsorption further suggests that valproate acted specifically on the lactate/pyruvate luminal transporter in the brush border membrane. This is reminiscent of the inhibition by valproate of the pyruvate mitochondrial transporter reported by Benavides et al [34]. In view of the striking amplitude of this effect, we suggest that urinary lactate might possibly be used to evaluate the compliance of patients taking this antiepileptic drug.

Metabolic effects of valproate

Abolishment of lactate and pyruvate oxidation. Valproate induced a marked reduction in renal lactate utilization, and even caused a net lactate production. This was accompanied by an increased concentration of renal cortical lactate. The reduction in lactate utilization could not be due to the inhibited reabsorption of lactate, as an increased cellular concentration of this anion followed the higher arterial concentration and cellular delivery through the basolateral membrane. At this site, lactate is transported into the renal cell by an anion exchanger which does not appear to be inhibited by valproate. The reduced oxidation of lactate and pyruvate could theoretically result from the following three mechanisms: 1) the observed cytosolic redox shift driving pyruvate into lactate; 2) an effect of valproate on the mitochondrial pyruvate transporter; 3) a decreased flux through mitochondrial pyruvate dehydrogenase (PDH).

Because tissue pyruvate concentration did not decrease significantly (mechanism 1), a redox-reduced fall in the pyruvate concentration below the Km of the mitochondrial transporter cannot explain its reduced oxidation. A valproate-induced inhibition of the mitochondrial transporter for pyruvate may be one explanation (mechanism 2); however, this mechanism alone would not account for this effect since the oxidation of the pyruvate generated in the mitosol was also reduced by

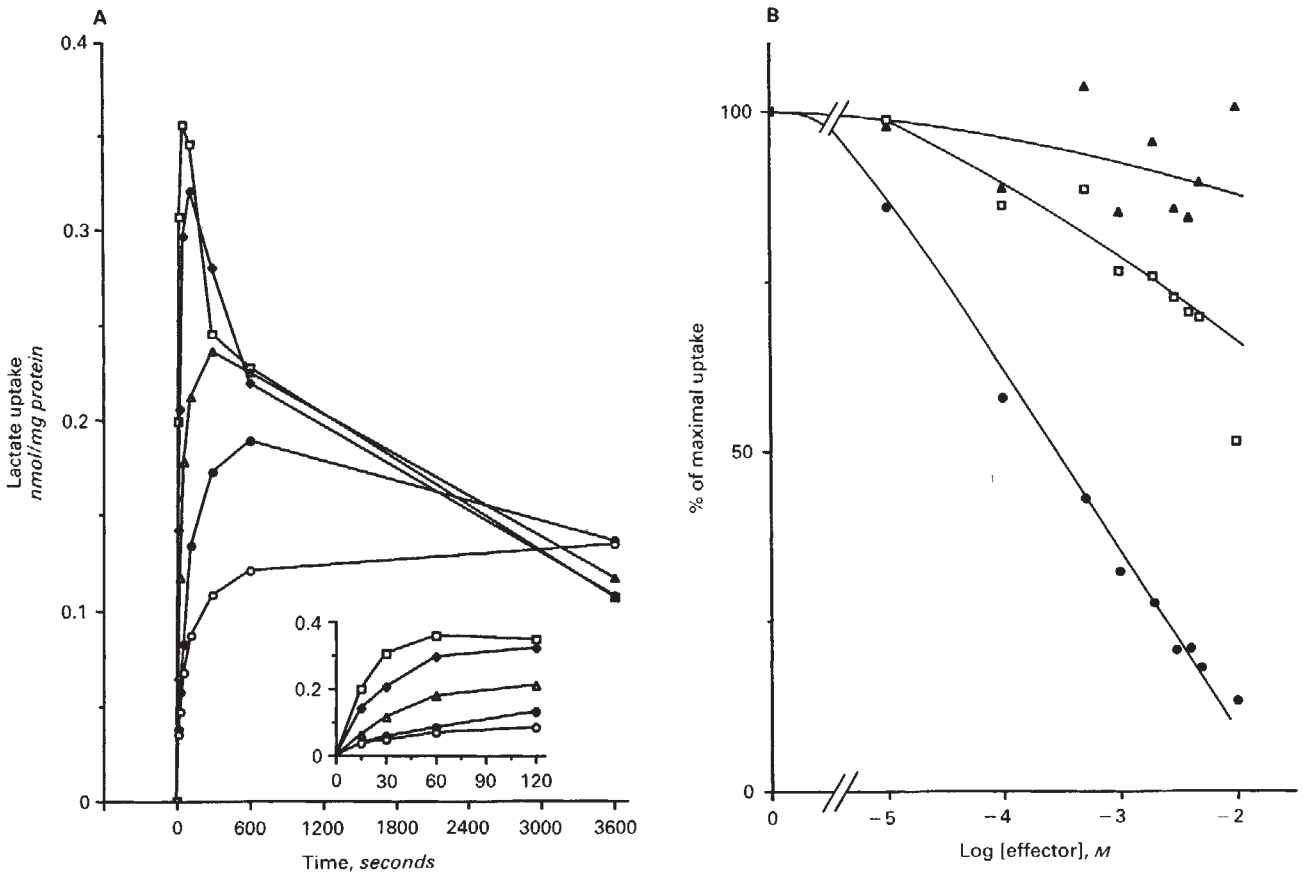

Fig. 4. A. *Accumulation of ^{14}C-L-lactate (40 μM) into vesicles of brush border membranes as a function of time.* In control situations as well as in the presence of valproate, H^+ and Na^+ gradients were created across the vesicle membranes by manipulation of the intra-/extravesicular medium composition: inside, pH 8.0 and 150 mM KCl, 0 mM NaCl; outside, pH 6.0 and 150 mM NaCl, 0 mM KCl. The "no gradient" situation was obtained by incubating the vesicles in intra-vesicular medium. The early kinetics of lactate accumulation is presented in the insert. Symbols are: (□) control, (◆) 0.1 mM VPA, (△) 1.0 mM VPA, (●) 5.0 mM VPA, (○) no gradient. **B.** Effect of progressive concentrations of valproate (●), acetate (□) and α-cyano-hydroxy-cinnamate (▲) on the accumulation of ^{14}C-lactate measured at 30 seconds. The results are expressed as % of the control (no effector) value.

valproate in vitro in proximal tubules [14] and thick ascending limbs [35], suggesting PDH inhibition. This latter effect (mechanism 3) might result from a direct effect of valproate on the PDH complex or from an increased phosphorylation of the enzyme secondary to the oxidation of valproate itself. It is of interest in this regard that the short-chain propionyl CoA and isovaleryl CoA are known to inhibit PDH activity in the pig kidney [36]. The production of valproyl CoA from valproate [37] could have the same inhibitory effect.

The decreased pyruvate oxidation did not lead to alanine synthesis because tissue pyruvate did not rise and because glutamate concentration decreased. The inhibition of pyruvate oxidation occurred in part in proximal tubules (refer to the metabolites profiles) but must also occur in the thick ascending limb and distal nephron. Indeed, in dogs with chronic metabolic acidosis, most of the lactate extracted by the kidney is oxidized by these distal segments of the nephron [28].

In view of the large reduction of renal lactate uptake, the unchanged oxygen consumption and overall renal transport observed in chronically acidotic dogs suggest a metabolic competition between valproate and lactate: the energy required for reabsorption was maintained. This evidence, together with similar observations obtained in vitro in renal tubules of proximal or distal origin [14], suggest that the metabolism of valproate itself may replace the oxidation of lactate/pyruvate. It is known that valproate can support cerebral mitochondrial respiration in vitro and serves as a source of NADH [38].

Accelerated conversion of glutamine into pyruvate. An accelerated flux between glutamine and oxaloacetate was induced by valproate as reflected by the marked reduction in the renal cortical concentrations of glutamine, glutamate and alphaketoglutarate. However the concentrations of oxaloacetate and malate did not rise secondarily, in sharp contrast with the increment observed when these fluxes were accelerated by acidosis [29]. The simultaneous decrease of oxaloacetate formation from pyruvate in valproate-treated dogs may explain this discrepancy if tissue oxaloacetate reflects a simultaneous production from glutamine and pyruvate. Indeed pyruvate carboxylase is an enzyme regulated in an allosteric fashion by acetyl CoA [39]. A valproyl CoA production exceeding its oxidation may decrease the tissue acetyl CoA [40]. A secondary reduction in pyruvate carboxylase activity can thus be expected and was suggested by the fall in the renal cortical concentra-

tions of malate and citrate (octanoate being able to protect the kidney against all these biochemical changes [37]).

In vivo, only acidotic animals demonstrated a modest stimulation of glutamine extraction and ammonia production. In contrast, in vitro, valproate administration markedly increased glutamine uptake and metabolism by both proximal tubules [14] and thick ascending limbs [35] at pH 7.4. Both situations thus contrast strikingly. This suggests that the in vivo situation somehow protects the kidney against the ammoniagenic effect of valproate. This may be related to the availability of endogenous free fatty acids in vivo [41, 42]. The fact that valproate and fatty acids have been reported to compete for the oxidation [43] may contribute to this latter effect. Indeed, valproate inhibits the oxidation of long-chain fatty acids probably through a reduced carnitine availability [44, 45], due to valproyl carnitine production, while octanoate reduces valproate oxidation.

Metabolism of valproate itself. Significant quantities of valproate can be delivered to the kidney and be metabolized in this organ [46, 47]. A significant renal metabolism of valproate is suggested by the marked difference observed between the total amount of valproate entering through the renal artery (valproate in arterial renal plasma flow plus continuous infusion) and the combined quantities measured in the renal vein and urine, the urinary excretion of unchanged valproate being minimal [48]. Furthermore, the change in the tissue redox potential, related to the infused load of valproate, also indicates a local metabolism which probably occurred both in the proximal tubules and in the distal nephron.

Indeed, sufficient β-oxidation of valproate, a reaction occurring in mitochondria [49], must have occurred to explain the reduction of the tissue NAD/NADH ratio. Because valproate induced a reduction and not an oxidation of the NAD/NADH ratio, valproate did not have in the kidney the uncoupling effect described in brain and liver mitochondria [50]. Other tissues than the kidney also metabolized valproate, since the arterial lactate/pyruvate ratio exceeded that observed in the kidney cortex.

Hyperammonemia. Despite the establishment of plasma concentrations of valproate much higher than the therapeutic plasma concentration around 0.5 mM [51], a modest hyperammonemia was observed following valproate administration in acidotic dogs only. Obviously, this effect did not result from a shift of ammonium from urine to renal venous blood since no urinary alkalinization, as observed with maleate [18, 52] and 4-pentenoate [53], occurred. Furthermore, the urinary excretion of ammonium probably rose because of the increased urinary excretion of lactate, acting as a poorly-reabsorbable organic anion. The increased renal ammonium delivery to the systemic blood resulting from chronic metabolic acidosis unmasked a reduced capacity for hepatic detoxification of ammonium following valproate administration. This suggests that valproate-induced hyperammonemia may be worsened by metabolic acidosis increasing renal ammonium delivery to the blood in presence of a valproate-induced reduced detoxification into urea. The absence of a significant renal ammoniagenic effect of valproate in the dog is in contrast to the data of Warter et al, who observed an increased release of ammonium into the renal vein and hyperammonemia both in the rat [12] and man [9, 10, 42]. Species differences between man, the rat and the dog could be responsible for this discrepancy.

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