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STIMULATION OF GLUTAMINE METABOLISM BY THE ANTIEPILEPTIC DRUG, SODIUM VALPROATE, IN ISOLATED DOG KIDNEY TUBULES

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Abstract—The effects of sodium valproate, a widely used antiepileptic drug and an hyperammonemic agent, on glutamine and glutamate metabolism were studied in isolated dog kidney tubules. Valproate markedly stimulated glutamine removal as well as the formation of ammonia, aspartate, pyruvate, lactate, alanine and glucose; the increase in ammonia formation was explained by a stimulation by valproate of flux not only through glutaminase (EC 3.5.1.2) but also through glutamate dehydrogenase (EC 1.4.1.3). By contrast, valproate did not stimulate glutamate removal or ammonia, aspartate and glucose formation from glutamate; this suggests that the increase in flux through glutamate dehydrogenase with glutamine as substrate was secondary to the increase in flux through glutaminase. Accumulation of pyruvate, alanine and lactate in the presence of valproate was much less from glutamate than from glutamine. Inhibition by amino-oxyacetate of accumulation of aspartate and alanine from glutamine caused by valproate did not prevent the acceleration of glutamine utilization and the subsequent stimulation of ammonia formation. These data are consistent with a stimulatory effect of valproate primarily exerted at the level of glutaminase in dog kidney tubules. However, the fact that assayed activity of glutaminase remained unchanged in the presence of valproate suggests that this compound accelerates flux through the latter enzyme by an indirect mechanism probably related to the renal metabolism of this compound.

Administration of sodium valproate (*n*-dipropylacetic acid, sodium salt), a widely used and very effective antiepileptic drug [1], often induces an hyperammonemia with extremely rare clinical signs of liver dysfunction [2–6]. This hyperammonemia has been first attributed to the inhibition by valproate of the urea cycle in the liver leading to a decrease in the hepatic removal of ammonia [7, 8]; such an explanation is based on experiments performed with isolated rat mitochondria [7, 8] or isolated rat hepatocytes [9] in which valproate was found to inhibit the synthesis of acetylglutamate, a well-established activator of carbamylphosphate synthetase, resulting in a decrease in the synthesis of urea from various precursors [9, 10].

However, experiments performed *in vivo* both in man and rat point to the kidneys as organs potentially responsible for the valproate-induced hyperammonemia [11, 12]; although the renal blood flow was not measured in these experiments, it was clearly shown that valproate increases both the negative arteriovenous difference for ammonia and the positive arteriovenous difference for glutamine across the human and rat kidney [11, 12]. In a recent paper [13], we have clearly established that indeed a high dose of valproate (200 mg/kg body wt) greatly stimulates the renal production of ammonia leading to a large increase in both the renal venous release and urinary excretion of this compound.

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This paper examines in isolated dog kidney-cortex tubules the possible mechanisms by which valproate stimulates the renal production of ammonia from glutamine, the main precursor of the ammonia produced by the dog kidney *in vivo* [14].

MATERIALS AND METHODS

Animals and preparation of isolated kidney-cortex tubules. Kidneys were from adult mongrel dogs (15–20 kg) of either sex fed on a standard diet (U.A.R., Villemoisson-sur-Orge, France). After the dogs were anesthetized with i.v. sodium penthiobarbital (25 mg/kg body wt), the kidneys were excised and placed in ice-cold isotonic saline. Kidney tubules were prepared by collagenase treatment as described previously [15].

Purity and viability of the tubules obtained were assessed by measurement of glucose synthesis from 5 mM L-lactate at rates linear with time and similar to those previously obtained [15].

Incubations. Incubations were performed at 37° in a shaking water-bath in 25 ml stoppered Erlenmeyer flasks in an atmosphere of O_2/CO_2 (19:1). Tubules were incubated in 4 ml of Krebs–Henseleit buffer (pH 7.40) with 5 mM L-glutamine or L-glutamate as substrate in the absence or the presence of valproate (sodium salt). The flasks were prepared in duplicate for all experimental conditions. Incubations were terminated by adding $HClO_4$ (final concn 2%, v/v). In all experiments, zero-time flasks were prepared with and without substrate by adding $HClO_4$ before the tubules. After removal of the denaturated protein by centrifugation (4000 *g* for 10 min), the supernatant was neutralized with 20% (w/v) KOH for metabolite determination.

Analytical methods. Lactate, pyruvate, glucose, alanine, aspartate, citrate, 2-oxoglutarate, fumarate, malate, glutamate, glutamine, ammonia, glycogen and also the dry weight of the amount of tubules added to the flasks were determined as previously described [15, 16].

Calculations. Net substrate utilization and product formation were calculated as the difference between the total contents of the flask (tissue + medium) at the start (zero-time flasks) and after 60 min of incubation. The metabolic rates are expressed in μmol of substance removed or produced/hr per g dry wt of tubule fragments. They are reported as means ± SE. The results were analysed by Student's *t*-test for paired data, comparing values obtained in the presence and the absence of valproate. In all cases, the criterion for statistical significance was $P < 0.05$.

The metabolic fate of the glutamine or glutamate added as substrate was established by calculating carbon and nitrogen balance. Complete oxidation of glutamine was calculated as the difference between the removal of glutamine and the sum of glutamate, glucose (expressed in C_3 units, because 2 molecules of glutamine are needed for the synthesis of each glucose molecule), lactate, pyruvate, alanine and aspartate found. Complete oxidation of glutamate was calculated as the difference between the removal of glutamate and the sum of glucose (expressed in C_3 units), lactate, pyruvate, alanine and aspartate found. With glutamine as substrate, flux through glutamate dehydrogenase was calculated as the difference between flux through glutaminase (taken as the removal of glutamine) and the accumulation of glutamate, aspartate and alanine; flux through 2-oxoglutarate dehydrogenase was calculated as the removal of glutamine minus the accumulation of glutamate. With glutamate as substrate, flux through glutamate dehydrogenase was taken as the glutamate removed minus the aspartate and alanine found and flux through 2-oxoglutarate dehydrogenase was taken as the glutamate removed.

Measurement of glutaminase activity. Glutaminase (EC 3.5.1.2) activity was measured by incubating for 30 min kidney tubules (42.7 ± 9.2 mg dry wt per flask; N = 4) as described above in the presence of 5 mM L-glutamine and 12.5 μM rotenone to prevent the metabolism of glutamate formed by glutaminase; glutaminase activity, taken as the net formation of glutamate (in μmol/g dry wt × 30 min) at the end of the incubation, was measured in the absence and the presence of valproate. In this assay, it was ascertained that glutamate formation was linear with time (up to 45 min) and with the amount of tubules added (up to 65 mg dry wt per flask); it was also verified that all the glutamine removed was accounted for by the formation of glutamate and that no synthesis of aspartate, alanine, glucose and of $^{14}CO_2$ from L-1-^{14}C-glutamine occurred.

Reagents. L-glutamate, L-glutamine, glutaminase (grade V), amino-oxyacetate and rotenone were supplied by Sigma Chemical Co. (St Louis, MO). Other enzymes and coenzymes came from Boehringer (Meylan, France). Sodium valproate was supplied by Sanofi Recherche (Service de Toxicologie, Montpellier, France). The other chemicals used were of analytical grade.

RESULTS

As shown in Table 1, valproate (0.01–10 mM) greatly stimulated glutamine removal by isolated dog kidney-cortex tubules; this effect was accompanied by a marked increase in the formation of ammonia, aspartate, alanine and lactate as well as in the accumulation of pyruvate. Glutamate accumulation was slightly stimulated except with 1 and 10 mM valproate; glucose synthesis from glutamine was also increased except with the lowest and the highest concentration of valproate (Table 1). No significant accumulation of intermediates of the tricarboxylic acid cycle or of glycogen was observed, indicating that the glutamine removed and not accounted for by the carbon products found was completely oxidized.

Carbon-balance calculations demonstrate that the carbon skeleton of the glutamine used was accounted for mainly by complete oxidation (41.1%) and the formation of glucose (30.8%) and glutamate (25.3%) in the absence of valproate (see Table 1); with increasing concentrations of valproate, the proportion of the glutamine carbon fragment removed that is accounted for by alanine, lactate, pyruvate and aspartate progressively increased, whereas complete oxidation expressed both in absolute terms or as percentage of the glutamine removed progressively decreased.

Calculations from the data of Table 1 also show that the increase in ammonia synthesis caused by valproate was greater than the increase in the glutamine removed, which means that valproate stimulated not only flux through glutaminase, which releases as ammonia the amide nitrogen of glutamine, but also flux through glutamate dehydrogenase, which releases as ammonia the amino nitrogen of glutamate formed from glutamine; as can be seen in Table 1, flux through glutamate dehydrogenase was indeed found to be increased by valproate but always to a much lesser extent than flux through glutaminase (taken as the glutamine removed).

In contrast with the finding with glutamine as substrate, no increase in glutamate removal was observed and no stimulation but rather an inhibition of the formation of ammonia and glucose occurred in the presence of valproate (Table 2); but addition of valproate caused a large increase in the accumulation of pyruvate and the formation of lactate and alanine. However this increase was less than that observed with glutamine as substrate. Aspartate formation from glutamate, which was very low, did not change in the presence of valproate, but complete oxidation of glutamate was inhibited by the highest concentrations of valproate used (Table 2).

With glutamine as substrate, the addition of valproate plus amino-oxyacetate, an inhibitor of transaminases [17], did not suppress the stimulation of glutamine removal and ammonia production despite a further increase in glutamate accumulation and an almost complete inhibition of aspartate and alanine synthesis (Table 3). Probably as a result of the inhibition of alanine caused by amino-oxyacetate, lactate accumulation from glutamine was greater in the presence of valproate plus amino-oxyacetate than in the presence of valproate alone. Calculations from Table 3 indicate that, despite a large increase in flux through glutamate dehydrogenase in the presence of

Table 1. Effect of valproate on the metabolism of 5 mM L-glutamine in dog kidney tubules

Experimental condition	Metabolite removal or production								Glutamine completely oxidized	Flux through glutamate dehydrogenase	Flux through 2-oxoglutarate dehydrogenase
	Glutamine	Glutamate	Ammonia	Aspartate	Alanine	Glucose	Pyruvate	Lactate			
Glutamine 5 mM	−481.0 ± 30.2	+121.8 ± 17.5	+866.3 ± 49.7	+5.4 ± 2.2	−0.6 ± 5.0	+74.1 ± 4.3	+4.0 ± 0.6	+4.4 ± 2.0	197.8 ± 26.3	354.4 ± 26.0	359.2 ± 23.3
Glutamine 5 mM + valproate 0.001 mM	−492.5 ± 34.5	+129.9 ± 19.1*	+875.4 ± 47.4	+8.0 ± 2.4*	+4.3 ± 6.1*	+78.3 ± 4.9	+4.7 ± 0.6*	+4.8 ± 2.1	184.2 ± 10.3	350.3 ± 17.8	362.6 ± 18.7
Glutamine 5 mM + valproate 0.01 mM	−710.1 ± 58.5*	+153.5 ± 22.1*	+1153.9 ± 84.7*	+26.2 ± 5.0*	+74.2 ± 25.1*	+114.6 ± 7.1*	+9.6 ± 2.4	+17.1 ± 7.6	200.3 ± 43.3	456.2 ± 48.3*	556.6 ± 43.5*
Glutamine 5 mM + valproate 0.1 mM	−826.5 ± 63.9*	+148.2 ± 24.2*	+1381.8 ± 157.5*	+36.2 ± 8.6*	+171.2 ± 25.8*	+122.5 ± 10.7*	+24.5 ± 4.7*	+61.0 ± 13.5*	140.4 ± 34.3*	470.9 ± 44.3*	678.3 ± 47.7*
Glutamine 5 mM + valproate 1 mM	−845.0 ± 64.7*	+141.9 ± 28.6	+1345.0 ± 85.6*	+42.8 ± 7.0*	+183.1 ± 19.9*	+101.0 ± 5.5*	+49.8 ± 4.1*	+117.3 ± 8.9*	108.1 ± 30.9*	477.2 ± 43.3*	703.1 ± 49.4*
Glutamine 5 mM + valproate 10 mM	−835.8 ± 61.4*	+112.7 ± 27.8	+1286.2 ± 86.6*	+53.3 ± 6.3*	+194.2 ± 15.6*	+83.0 ± 6.5	+79.9 ± 8.0*	+147.9 ± 11.1*	81.8 ± 26.5*	475.6 ± 45.5*	723.1 ± 52.7*
No substrate	−5.5 ± 2.9	−27.7 ± 5.8	+192.0 ± 13.8	−0.5 ± 1.8	−14.7 ± 2.4	+14.2 ± 1.1	+1.7 ± 0.8	−0.8 ± 1.1	—	—	—
No substrate + valproate 10 mM	−5.3 ± 3.0	−32.1 ± 6.3*	+183.8 ± 24.3	−4.6 ± 1.5	−10.6 ± 2.9*	+2.3 ± 0.5*	+2.7 ± 0.2	+17.9 ± 1.4*	—	—	—

Kidney tubules (22.5 ± 3.5 mg dry wt per flask) were incubated for 60 min as described in the Methods section. Results (μmol/g dry wt) for metabolite removal (−) or production (+) are reported as means ± SE for 4 experiments performed in duplicate. Statistical difference was measured by the paired Student's *t*-test against the control without valproate: * $P < 0.05$.

Table 2. Effect of valproate on the metabolism of 5 mM L-glutamate in dog kidney tubules

Experimental condition	Metabolite removal or production							Glutamate completely oxidized	Flux through glutamate dehydrogenase	Flux through 2-oxoglutarate dehydrogenase
	Glutamate	Ammonia	Aspartate	Alanine	Glucose	Pyruvate	Lactate			
Glutamate 5 mM	−284.9 ± 15.5	+361.2 ± 8.6	+3.6 ± 3.7	+0.1 ± 3.5	+66.1 ± 2.5	+2.5 ± 0.5	+1.9 ± 0.7	144.6 ± 15.2	281.2 ± 13.1	284.9 ± 15.5
Glutamate 5 mM + valproate 0.001 mM	−279.6 ± 18.6	+363.8 ± 12.6	+6.4 ± 5.5	+0.7 ± 3.6	+66.8 ± 2.6	+2.0 ± 0.2	+1.6 ± 0.4	135.3 ± 19.5	272.5 ± 15.6	279.6 ± 18.6
Glutamate 5 mM + valproate 0.01 mM	−269.9 ± 12.9	+359.8 ± 5.2	+9.5 ± 8.3	+4.0 ± 2.4	+66.2 ± 2.4	+2.3 ± 0.2	+1.2 ± 0.9	120.5 ± 7.0	256.4 ± 6.5	269.9 ± 12.9
Glutamate 5 mM + valproate 0.1 mM	−292.5 ± 14.1	+344.3 ± 7.0	+6.8 ± 4.5	+32.0 ± 4.9*	+58.8 ± 2.4*	+5.4 ± 0.8*	+8.5 ± 1.9*	122.2 ± 10.3	253.7 ± 6.4	292.5 ± 14.1
Glutamate 5 mM + valproate 1 mM	−263.1 ± 17.2	+327.6 ± 8.0*	+9.0 ± 11.0	+50.0 ± 2.6*	+40.1 ± 1.5*	+13.9 ± 0.6*	+31.7 ± 2.9*	78.3 ± 8.9*	204.1 ± 6.5*	263.1 ± 17.2
Glutamate 5 mM + valproate 10 mM	−304.1 ± 8.3	+303.7 ± 12.1*	+2.5 ± 3.1	+79.0 ± 2.8	+24.7 ± 2.4*	+49.1 ± 2.7*	+57.9 ± 1.7*	66.2 ± 2.9*	222.6 ± 4.1*	304.1 ± 8.3
No substrate	−29.3 ± 2.3	+197.6 ± 2.6	−17.5 ± 1.3	−11.4 ± 1.5	+13.2 ± 2.6	+0.5 ± 0.2	−0.5 ± 0.4	—	—	—

Kidney tubules (23.3 ± 1.3 mg dry wt per flask) were incubated for 60 min as described in the Methods section. Results (μmol/g dry wt) for metabolite removal (−) or production (+) are reported as means ± SE for 4 experiments performed in duplicate. Statistical difference was measured by the paired Student's *t*-test against the control without valproate: * $P < 0.05$.

Table 3. Effect of amino-oxyacetate on the stimulation of the metabolism of 5 mM L-glutamine by valproate in dog kidney tubules

Experimental condition	Metabolite removal or production							
	Glutamine	Glutamate	Ammonia	Aspartate	Alanine	Glucose	Pyruvate	Lactate
Glutamine 5 mM	−479.3 ± 40.5	+150.9 ± 21.0	+803.3 ± 57.1	+13.6 ± 6.5	+30.3 ± 9.0	+50.1 ± 3.9	+14.2 ± 4.1	+11.1 ± 3.4
Glutamine 5 mM + valproate 0.1 mM	−752.3 ± 52.9*	+188.7 ± 23.9*	+1181.0 ± 88.7*	+101.6 ± 15.2*	+134.6 ± 8.7*	+78.3 ± 4.9*	+36.3 ± 3.8*	+66.9 ± 4.9*
Glutamine 5 mM + valproate 0.1 mM + amino-oxyacetate 1 mM	−709.9 ± 58.2*	+210.9 ± 25.6*	+1199.5 ± 84.7*	+15.6 ± 2.6	+4.2 ± 1.1*	+37.1 ± 2.9*	—	+108.7 ± 9.7*
Glutamine 5 mM + amino-oxyacetate 1 mM	−464.0 ± 22.6	+148.2 ± 20.8	+833.1 ± 75.3	+4.2 ± 0.3	+3.9 ± 1.3*	+30.6 ± 4.3*	—	+33.5 ± 4.7*
No substrate	−5.1 ± 0.3	−26.1 ± 4.1	+147.4 ± 10.8	−17.2 ± 4.1	−11.7 ± 1.1	+9.0 ± 0.8	+0.5 ± 0.5	−1.2 ± 1.4
No substrate + amino-oxyacetate 1 mM	−3.6 ± 2.9	−40.8 ± 4.3*	+115.3 ± 6.4*	−10.6 ± 3.7*	+9.7 ± 0.5*	+5.2 ± 0.3*	—	+6.6 ± 2.6

Kidney tubules (15.1 ± 2.3 mg dry wt per flask) were incubated for 60 min as described in the Methods section. Results (μmol/g dry wt) for metabolite removal (−) or production (+) are reported as means ± SE for 4 experiments performed in duplicate. Pyruvate could not be assayed in the presence of amino-oxyacetate. Statistical difference was measured by the paired Student's *t*-test against the control without valproate or amino-oxyacetate: * $P < 0.05$.

valproate plus amino-oxyacetate, glucose synthesis from glutamine did not return to the levels observed in the presence of valproate (Table 3).

In the absence of valproate the activity of glutaminase in dog kidney tubules was found to be 101.6 μmol/g dry wt per 30 min; addition of 0.1 and 1 mM valproate did not alter this activity which was 98.4 ± 9.6 and 102.8 ± 10.4 μmol/g dry wt per 30 min, respectively. This means that valproate had no direct stimulatory effect on renal glutaminase kept in its normal intracellular environment in isolated dog kidney tubules (N = 4).

DISCUSSION

Our results clearly demonstrate that valproate stimulated the removal of glutamine and the production of ammonia by dog kidney tubules. Increased glutamine removal may have resulted from a stimulatory effect of valproate on glutaminase and/or from an augmented removal of glutamate (synthezised from glutamine via glutaminase), a potent end-product inhibitor of renal glutaminase (EC 3.5.1.2) [18]. In the presence of low concentrations of valproate (0.01 and 0.1 mM), increased accumulation of glutamate from glutamine, despite a considerable increase in glutamate metabolism via glutamate dehydrogenase, alanine and aspartate aminotransferase (see Table 1), strongly suggests that this compound stimulates glutamine removal not only by increasing glutamate metabolism but also by stimulating glutaminase.

Additional evidence that stimulatory effect of valproate is exerted primarily on glutaminase was obtained by the addition of amino-oxyacetate, an inhibitor of transaminases [17], which almost completely suppressed the accumulation of alanine and aspartate caused by valproate without abolishing the large stimulation of glutamine removal (Table 3). It should be noted that the latter observation rules out the possible involvement of glutamine-pyruvate transaminase and glutamine-oxaloacetate transaminase in the stimulation of glutamine utilization observed in the presence of valproate.

Flux through glutamate dehydrogenase was augmented by valproate when glutamine was the substrate but remained unaltered or even decreased when glutamate was the substrate (Tables 1 and 2). This clearly indicates that, with glutamine as substrate (Table 1), increased flux through glutamate dehydrogenase was secondary to the stimulation of flux through glutaminase leading to an increased synthesis, metabolism, and (under some conditions) accumulation of glutamate. Since the pathway for the conversion of glutamine into glucose necessarily involves glutamate dehydrogenase, the increase in glucose synthesis, which paralleled the increase in flux through glutamate dehydrogenase (Table 1), was therefore due to stimulation by valproate of flux through glutaminase and, thus, of the entire pathway between glutamine and glucose.

It should be stressed that the stimulation of ammonia synthesis from glutamine was due in great part to the stimulation by valproate of flux through glutaminase which was, under all conditions, higher

than the stimulation of flux through glutamate dehydrogenase (see Tables 1 and 3).

Increased synthesis of alanine from both glutamine and glutamate in the presence of valproate (Tables 1–3) clearly means that valproate increased the availability of pyruvate for the alanine aminotransferase reaction; increased availability of pyruvate was also revealed by increased accumulation of pyruvate and lactate in the presence of valproate (Tables 1–3). Such an accumulation of pyruvate, which may result from an inhibition by valproate either of pyruvate transport into the mitochondria or of pyruvate metabolism by pyruvate dehydrogenase, probably explains why valproate reduced complete oxidation of glutamine and glutamate (Tables 1 and 2). With the same concentration of valproate, the fact that the stimulation of alanine accumulation is always much greater from glutamine than from glutamate may be surprising since glutamate is a substrate of the alanine aminotransferase reaction. This is possibly due to the very large stimulation of pyruvate synthesis from glutamine (calculated as the glutamine completely oxidized plus the pyruvate, lactate and alanine found) which resulted from the stimulation of flux through glutaminase.

It is interesting to note that the same concentrations of valproate increased the formation of aspartate, and therefore the availability of oxaloacetate for the aspartate aminotransferase reaction, when glutamine but not glutamate, was the substrate (Table 1–3). This can be explained by the fact that the synthesis of oxaloacetate is greater from glutamine than from glutamate in the presence of valproate; as shown by the data in Tables 1 and 2, flux through 2-oxoglutarate dehydrogenase (an estimate of the maximal capacity for synthesizing oxaloacetate from glutamine or glutamate) was in the same range from either glutamine or glutamate in the absence of valproate, but this flux was doubled in the presence of valproate only when glutamine was the substrate. Beside suggesting that the utilization of oxaloacetate, presumably by phosphoenolpyruvate carboxykinase, was a rate-limiting step when glutamine metabolism was stimulated, this observation provides further evidence that valproate had a primary effect at an enzymatic step specific to glutamine metabolism, namely glutaminase.

Inhibition of glucose synthesis from glutamate observed in the presence of valproate was probably due to the reduction of flux through glutamate dehydrogenase (Table 2) secondary to diversion of glutamate from oxidative deamination to transamination with pyruvate; it is also conceivable that the increase in lactate synthesis secondary to pyruvate accumulation competed with the gluconeogenic pathway for the reducing equivalents available in the cytosol.

It should be emphasized that valproate had no direct stimulatory effect on the activity of glutaminase assayed in dog kidney tubules despite its very large stimulation of flux through glutaminase in intact functioning tubules. This suggests that valproate exerted the latter effect by an indirect mechanism which remains to be elucidated. In this respect, it should be pointed out that valproate has been shown to be metabolized by rat kidney tubules [19]. Preliminary experiments performed in our laboratory indicate that this is also the case in dog kidney tubules. This raises the question of whether the stimulation of glutamine metabolism found in our study is a general effect due to all organic acids possibly metabolized by renal tubules. When dog tubules (N = 4; 22.6 ± 1.2 mg dry wt per flask) were incubated with 5 mM L-glutamine for 60 min, glutamine removal was inhibited by 36.6 and 20.4% in the presence of 1 mM sodium octanoate and 1 mM sodium pentanoate, respectively; under the latter conditions, the production of ammonia was reduced by 48.6 and 35.5%, respectively; in the same experimental series, addition of 1 mM sodium valproate stimulated glutamine removal by 66.1% and ammonia production by 48.9% (N = 4; results not shown). In another experimental series (N = 4; results not shown), the effect of octanoate was found to be dose-dependent. For example, inhibition of the removal of 5 mM L-glutamine by 0.5 mM octanoate was 53.3% that observed with 1 mM octanoate; similarly inhibition by 0.5 mM octanoate of ammonia production from 5 mM L-glutamine was 60.8% that found with 1 mM octanoate. Finally, flux through glutamate dehydrogenase, taken as the glutamine removed minus the glutamate accumulated, was reduced by 51.3% with 0.5 mM octanoate, and was completely abolished in the presence of 1 mM octanoate.

These findings clearly indicate that the stimulation of glutamine metabolism observed in the present study is specific to valproate. Since valproate appears to be metabolized by the tubules, it seems important to establish the nature of valproate metabolites possibly accumulated during such metabolism. This will enable us to test whether one (or several) valproate metabolites formed by dog tubules is directly responsible at the enzymatic level for the stimulation of renal glutaminase. Studies along these lines are underway in our laboratory.

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