Effect of Valproate on Lactate and Glutamine Metabolism by Rat Renal Cortical Tubules (42872)

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Abstract. **The metabolic effects of sodium valproate (VPA) on rat renal cortical tubules have been examined. When 1 or 5 m*M* lactate was used as substrate in the incubation medium, VPA decreased markedly the lactate uptake by the tubules. When 1 or 5 m*M* glutamine was used, the addition of VPA accelerated glutamine uptake, ammoniagenesis, but also stimulated markedly the accumulation of lactate and pyruvate produced from glutamine. VPA had a dose-dependent inhibitory effect on gluconeogenesis from both glutamine and lactate. With 5 m*M* glutamine, VPA also induced a significant accumulation of glutamate in the medium. The oxygen consumption by the tubules was diminished by 40% following VPA addition. It is concluded that VPA modifies the metabolism of rat cortical tubules by interfering with the oxidation of natural substrates and stimulates in this fashion the production of ammonia by kidney tubules.**

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Sodium valproate (2-propylpentanoate) is an effective and widely used anticonvulsant (1). Valproate (VPA) administration may result in an increased blood concentration of ammonia, rarely associated with clinical manifestations (2–7). This adverse effect of VPA has been attributed to a reduced hepatic conversion of ammonia into urea (2–5, 7–9). Valproate also inhibits fatty acids and pyruvate oxidation, fatty acids synthesis, and gluconeogenesis in isolated rat hepatocytes (10–12) and may even cause hepatic injuries (5, 13, 14). Another factor contributing to this hyperammonemia could be an increased release of ammonia by extrahepatic tissues. Indeed, arteriovenous measurements across the human (6) and rat (15) kidney have shown that VPA accelerates the renal glutamine metabolism, resulting in an increased release of ammonia in the renal venous blood. *In vitro*, it has also been reported that VPA accelerates glutamine metabolism by rat (16) and dog (17) renal cortical tubules. VPA also decreases lactate utilization by dog cortical tubules (17) as well as by dog thick ascending limbs (18) *in vitro* and by the dog kidney *in vivo* (19).

The site of the metabolic effects of VPA on renal metabolism has not been explored as thoroughly as that in the brain or liver (1, 20). The present study was thus undertaken to examine the metabolic effect of acute exposure to VPA on rat renal cortical tubules in suspension *in vitro*. Our data demonstrate that VPA accelerates glutamine utilization and ammonia production but reduces the oxidation of pyruvate and gluconeogenesis from both lactate and glutamine. Our data suggest that VPA competes for oxidation with natural substrates of the kidney. The effects of VPA observed in this study on rat tubules are remarkably similar to those described with a similar preparation from the dog kidney (17).

Materials and Methods

Acute Exposition of Tubules to Valproate. Six experiments were performed on Sprague-Dawley rats weighing 360–400 g. The rats were maintained on a commercial diet and had free access to water. Animals were fasted 24 hr before the experiments. On the morning of each experiment, eight rats were anesthetized with pentobarbital (6 mg/100 g body wt) administered intraperitoneally and the abdominal cavity was opened. The kidneys were removed and placed in 50 ml of ice-cold Krebs-Henseleit saline (pH 7.4) previously gassed at 20°C with 95% O_2:5% CO_2 for 30 min. After decap-

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sulation, the kidneys were cut in half and the outer medulla was carefully dissected out. The resultant chips of kidney cortex were sliced with a Stadie-Riggs microtome. The slices were pooled and used for preparation of cortical tubules as described previously (17). At the end of the procedure, the tubules were resuspended in six volumes of Krebs-Henseliet saline in order to obtain a yellowish suspension containing about 60 mg/ml of cortical tubules. Each suspension was examined under the microscope, and the viability was assessed by exclusion of trypan blue. In each case, less than 15% of the structures observed were taking up the dye. Approximately 30 mg wet wt of renal cortical tubules when incubated in a final volume of 4 ml of gassed Krebs-Henseleit saline adjusted at pH 7.4 according to the following experimental protocol.

Experimental Protocol. The experimental protocol used allowed us to study the effect of various concentrations of VPA on tubules metabolizing different substrates. The incubation medium contained either 0, 0.1, 1, or 10 m*M* VPA neutralized to pH 7.4. For each level of VPA, the substrate placed in the incubation medium was either 1 or 5 m*M* L-glutamine (together with 0.1 or 0.5 m*M* L-glutamate, respectively) or 1 or 5 m*M* L-lactate (with 0.1 or 0.5 m*M* pyruvate, respectively). The combination of substrates glutamine + lactate was also studied. At the end of the incubation procedure, the flask content was deproteinized with 0.5 ml of 40% w/v perchloric acid. A 30-min incubation period was used when 1 m*M* substrate was presented to the tubules and a 60-min incubation when the substrates were at 5 m*M* in order to avoid the exhaustion of all exogenous substrates during the incubation (with 1 m*M*), and to magnify the metabolic changes measured during incubation (with 5 m*M*). All results were nevertheless expressed in μmoles/g wet wt/hr. Glutamine, glutamate, ammonia, aspartate, alanine, lactate, pyruvate, glucose, citrate, and malate were measured by enzymatic techniques on a neutralized aliquot of the perchlorate extract as reported elsewhere (21, 22). Valproate was also measured by a fluorescence polarization technique (23).

Oxygen consumption by the tubules was also measured at 37°C using a Clark oxygen electrode fitted to a closed chamber of domestic design (24). Aliquots (0.5 ml) of the tubule suspensions, diluted to obtain a final concentration of 10 mg/ml, were heated to 37°C (5 min) and introduced in the 3.6-ml oxymetric chamber containing Krebs-Henseleit saline at pH 7.4 and the appropriate substrates. The change in partial pressure of oxygen (PO_2) in the chamber was then followed as a function of time. Oxygen consumption by tubules was linear until a very low PO_2 was reached (in about 15 min). Each measurement of oxygen consumption integrated the change of PO_2 observed during a 10-min observation time. It was assumed that the rate observed over 10 min was representative of this process occurring during up to 60 min in a flask.

Calculations. The major metabolic rates for glutamine (gluconeogenesis, oxidation, amino acids, organic acids) were estimated using the assumption that this amino acid was the major source of metabolites (carbon and nitrogen), accumulating in the flask when glutamine was added alone in the incubation medium, as described by Vinay *et al.* (25). Similar calculations were performed when lactate or the combination lactate + glutamine was used. In the latter case, the sum of substrates extracted was compared with the sum of metabolites produced in order to estimate the maximal oxidation of these substrates combined. The oxidation of CO_2, being estimated by the net disappearance of substrates from the flasks, also includes the accumulation of nonmeasured organic acids. Alanine production was constant in all studied conditions and was therefore not considered as originating from lactate or glutamine metabolism.

Statistical Analysis. The statistical analysis of the data was made using variance analysis for repeated measurements followed by comparisons of all means by the Sheffé procedure. The comparisons were considered significant when the P was less than 0.01.

Results

Effects of Valproate on the Metabolism of Rat Renal Cortical Tubules Incubated with Lactate and/or Glutamine. *Lactate, 1 and 5 mM (Table I).* In the absence of VPA, lactate and pyruvate were taken up by the tubules at the rate expected from fasted rats' cortical tubules (25). A small accumulation of glutamine, glutamate, and alanine was observed. A small production of NH_4 is detected, indicating a limited utilization of endogenous amino acids. This latter phenomenon was significantly reduced by using 5 m*M* lactate. At this latter concentration, lactate and pyruvate are extracted significantly more than with 1 m*M*. Glucose production is a significant metabolic rate for the lactate extracted at both concentrations and proceeded more rapidly with 5 m*M* than with 1 m*M* lactate.

If we assume that most of the metabolites appearing in the flask during the incubation period are directly or indirectly derived from the exogenous substrates presented to the tubules (10:1 lactate and pyruvate), it is possible to calculate that around 42% of the measured uptake of those intermediates completely disappears from the flask, presumably undergoing net oxidation to CO_2. This latter process is stimulated by 5 m*M* lactate as compared with 1 m*M* lactate. It thus appears that, in the absence of VPA, kidney tubules of fasted rats are able to metabolize lactate rapidly toward gluconeogenesis and oxidation, both processes being unsaturated when only 1 m*M* initial lactate concentration is provided in the incubation medium.

Table I. Effect of Valproate on the Metabolism of Lactate by Renal Cortical Tubules of Fasted Rats[a]

	VPA			
	0	0.1 m*M*	1 m*M*	10 m*M*
1 m*M* lactate/0.1 m*M* pyruvate				
Lactate	−113.8 ± 5.7	−98.3 ± 8.7	−60.5 ± 5.1*	−42.0 ± 5.4*
Pyruvate	−8.2 ± 1.9	−6.2 ± 1.4	−5.3 ± 2.6	−9.3 ± 2.2
Malate	0.5 ± 0.3	0.2 ± 0.3	−0.3 ± 0.2	−0.5 ± 0.2
Glucose	28.4 ± 1.5	17.8 ± 2.9*	5.5 ± 1.0*	2.1 ± 0.5*
Citrate	−0.8 ± 0.8	−0.3 ± 1.1	−0.3 ± 0.9	−1.5 ± 0.4
Glutamine	3.2 ± 0.9	1.0 ± 0.9	−0.3 ± 1.1	−0.6 ± 0.8
Glutamate	6.0 ± 2.7	5.3 ± 3.1	4.2 ± 3.1	1.1 ± 1.9
Alanine	4.7 ± 1.2	4.5 ± 1.1	4.4 ± 1.2	3.5 ± 1.3
Ammonia	15.7 ± 3.3	19.4 ± 2.2	21.6 ± 2.9	21.5 ± 4.0
Valproate	NM	NM	NM	−1008 ± 72
Metabolic rates				
To glucose	56.8 (47%)	35.6 (34%)	11.0 (17%)	4.2 (8%)
To CO_2 + unmeasured metabolites	51.6 (42%)	58.2 (56%)	47.1 (72%)	45.1 (88%)
5 m*M* lactate/0.5 m*M* pyruvate				
Lactate	−175.4 ± 13.1	−161.8 ± 21.5	−104.4 ± 12.4	−78.0 ± 10.5*
Pyruvate	−39.3 ± 1.3	−32.2 ± 2.2	−23.6 ± 2.1	−9.3 ± 2.1*
Malate	2.0 ± 0.4	1.4 ± 0.4	0.3 ± 0.2	−0.2 ± 0.1
Glucose	38.0 ± 3.0	29.0 ± 3.0	7.0 ± 1.2*	4.5 ± 0.7*
Citrate	−0.3 ± 0.9	−0.7 ± 1.2	−1.3 ± 0.9	1.5 ± 0.8
Glutamine	2.1 ± 0.4	0.4 ± 0.4*	0.3 ± 0.2*	0.3 ± 0.3*
Glutamate	2.0 ± 0.2	1.9 ± 0.3	0.5 ± 0.3*	0.3 ± 0.2*
Alanine	3.3 ± 0.4	4.2 ± 0.6	3.4 ± 0.2	3.7 ± 0.8
Ammonia	8.5 ± 1.0	11.9 ± 0.8	14.9 ± 0.8*	17.0 ± 2.2*
Valproate	NM	NM	NM	−659 ± 112
Metabolic rates				
To glucose	76.0 (35%)	58.0 (30%)	14.0 (11%)	9.0 (10%)
To CO_2 + unmeasured metabolites	129.6 (60%)	128.8 (66%)	110.8 (86%)	75.7 (87%)

[a] Data refer to net production (+) or utilization (−) of metabolites in μmol/g wet wt^{-1} hr^{-1}. Mean ± SEM (six experiments). NM, not measured. * $P < 0.01$ control vs VPA. Analysis of variance for repeated measurements (performed on measured data only). The maximal oxidation estimated from the carbon balance (omitting alanine) and the glucose production are presented along with the percentage of distribution of the lactate + pyruvate metabolized through these pathways.

When valproate concentration is progressively increased in the flask from 0.1 to 10 m*M*, the following observations are made. First, the extraction of lactate is significantly reduced in a manner proportional to the amount of valproate added. This is mainly related to a striking reduction in the glucose production as well as to a more modest reduction of apparent lactate oxidation. Simultaneously, a significant diminution of the small glutamine synthesis (which is reversed into net glutamine utilization) and of glutamate synthesis is observed. No significant change in alanine production is demonstrated. When valproate was measured in the flask, a 50% fall in the VPA concentration was simultaneously observed, indicating that VPA itself is metabolized by rat cortical tubules. VPA metabolism is significantly reduced when the exogenous lactate is raised from 1 to 5 m*M*.

Metabolism of glutamine, 1 and 5 mM (Table II). When 1 m*M* glutamine (plus 0.1 m*M* glutamate) is presented to cortical tubules, the following observations are made. First, glutamine is significantly extracted and supports a gluconeogenic flux of a magnitude similar to that observed with lactate. An accumulation of glutamate is observed. The amount of alanine appearing in the flask is identical to that observed with lactate. Since glutamine is an aminated molecule, the metabolic rate of glutamine can be in part estimated by following the appearance of amino acids and ammonium in the flask. It is first demonstrated that virtually all of the nitrogen present in the extracted glutamine molecules can be recovered as amino acids and ammonium (1 or 5 m*M* glutamine). The ammonium production unexplained by glucose, organic acids, or amino acids accumulation in the flask can be taken as an indirect indication of the maximal glutamine oxidation (plus accumulation of unmeasured organic acids). This value is calculated as 11.9 $\mu mol/g^{-1} \cdot h^{-1}$ with 1 m*M* glutamine but up to 39.6 with 5 m*M* glutamine. The calculated carbon and ammonium balances provide a very similar number for the apparent oxidative metabolic

Table II. Effect of Valproate on the Metabolism of Glutamine by Renal Cortical Tubules of Fasted Rats[a]

	VPA			
	0	0.1 m*M*	1 m*M*	10 m*M*
1 m*M* glutamine/0.1 m*M* glutamate				
Glutamine	−83.8 ± 4.2	−109.6 ± 6.4*	−121.5 ± 6.0*	−122.4 ± 5.9*
Glutamate	11.1 ± 4.4	18.6 ± 3.6	17.3 ± 3.4	14.7 ± 3.8
Lactate	2.4 ± 0.7	5.3 ± 0.4	13.4 ± 1.2*	15.4 ± 1.4*
Pyruvate	1.4 ± 0.2	2.6 ± 0.2*	6.0 ± 0.4*	10.0 ± 0.7*
Malate	−0.2 ± 0.2	0.2 ± 0.1	0.6 ± 0.1	0.1 ± 0.2
Glucose	30.3 ± 2.1	33.0 ± 2.6	23.0 ± 3.0*	20.7 ± 1.9*
Citrate	−0.9 ± 0.3	−0.6 ± 0.2	−1.2 ± 0.6	−1.2 ± 0.7
Aspartate	0.5 ± 1.4	1.4 ± 2.3	0.6 ± 2.6	1.0 ± 1.6
Alanine	3.5 ± 1.1	4.1 ± 1.2	4.7 ± 1.2	4.9 ± 1.1
Ammonia	162.0 ± 7.1	216.2 ± 10.1*	236.9 ± 10.4*	244.8 ± 12.9*
Valproate	NM	NM	NM	−959 ± 91
Metabolic rates				
To glucose	60.6 (72%)	66.0 (60%)	46.0 (38%)	41.4 (34%)
To CO_2 + unmeasured metabolites				
From carbon balance	5.4 (6%)	12.1 (11%)	34.1 (28%)	36.1 (29%)
From NH_4 balance	11.9 (14%)	24.6 (22%)	44.7 (37%)	48.9 (40%)
To amino acids (Glu + Asp)	11.6 (14%)	20.0 (18%)	17.9 (15%)	15.7 (13%)
To measured organic acids	2.7 (3%)	7.5 (7%)	18.8 (15%)	24.3 (20%)
5 m*M* glutamine/0.5 m*M* glutamate				
Glutamine	−195.7 ± 11.3	−235.6 ± 9.6	−259.4 ± 16.3*	−253.6 ± 18.8
Glutamate	48.3 ± 5.9	60.7 ± 2.9	70.8 ± 3.5*	65.4 ± 5.2*
Lactate	4.8 ± 0.4	11.3 ± 1.5	22.0 ± 1.8*	24.7 ± 2.3*
Pyruvate	2.0 ± 0.2	3.3 ± 0.3*	6.0 ± 0.4*	8.1 ± 0.5*
Malate	0.6 ± 0.2	0.8 ± 0.1	2.0 ± 0.3	1.0 ± 0.2
Glucose	43.9 ± 3.1	47.5 ± 3.6	34.8 ± 5.5	27.9 ± 3.2*
Citrate	0.1 ± 0.2	−0.1 ± 0.1	−0.3 ± 0.3	1.0 ± 0.6
Aspartate	10.8 ± 2.0	13.0 ± 1.4	13.7 ± 2.7	14.5 ± 2.1
Alanine	4.0 ± 1.0	4.0 ± 0.8	5.0 ± 0.9	5.0 ± 1.0
Ammonia	328.9 ± 19.5	378.3 ± 16.4	396.3 ± 24.5	384.0 ± 28.8
Valproate	NM	NM	NM	−893 ± 88
Metabolic rates				
To glucose	87.8 (45%)	95.0 (40%)	69.6 (27%)	55.8 (22%)
To CO_2 + unmeasured metabolites				
From carbon balance	37.3 (19%)	51.6 (22%)	66.1 (26%)	78.1 (31%)
From NH_4 balance	39.6 (20%)	42.0 (18%)	56.6 (22%)	61.5 (24%)
To amino acids (Glu + Asp)	59.1 (30%)	73.7 (31%)	84.5 (33%)	79.9 (32%)
To measured organic acids	7.5 (4%)	15.3 (6%)	29.7 (12%)	34.8 (14%)

[a] Data refer to net production (+) or utilization (−) of metabolites in μmol/g wet wt^{-1} hr^{-1}. Mean ± SEM (six experiments). NM, not measured. * $P < 0.01$ control vs VPA. Analysis of variance for repeated measurements (performed on measured data only). The maximal oxidation estimated from the carbon balance (omitting alanine) and the ammonium balance as well as the observed glucose production and accumulation of organic acids are presented along with the percentage of distribution of glutamine metabolized through these pathways.

rate. Thus, in the absence of valproate, kidney tubules are able to metabolize glutamine efficiently in accord with previous reports (25).

When valproate is progressively added in the flasks, the extraction of glutamine is significantly increased in contrast to the observation made with lactate. This is in part due to an accumulation of glutamate and organic acids (mainly lactate and pyruvate) in the flask and occurs despite a significant reduction in glucose synthesis. This latter process is in fact slightly stimulated at the lowest concentration of VPA (0.1 m*M*), whereas it is significantly decreased with 1 and 10 m*M* VPA. The carbon and ammonium balances disclose that an increasing amount of the glutamine carbon skeleton is not recovered in the measured metabolites, giving the impression that the net oxidation of glutamine is stimulated. This is probably not so, since net oxidation of glutamine proceeds through pyruvate formation, and since we have observed that pyruvate accumulation occurs upon exposure to VPA. Thus, we must conclude that accumulation of unmeasured organic acids (including α-ketoglutarate and succinate) takes place in our experiments when VPA is added to tubules metabolizing glutamine (but not lactate). Again, the appearance of ammonium in the flask confirms the carbon balance. VPA is itself largely metabolized, as described

above, and VPA oxidation is reduced by the provision of alternative substrates (5 m*M* glutamine as compared with 1 m*M* glutamine).

Metabolism of lactate + glutamine (Table III). When lactate + glutamine are provided together to cortical tubules in the absence of VPA, the extraction of each substrate occurs at a lower rate than what is observed when each one is presented alone to the tubules. With 1 m*M* concentration, gluconeogenesis proceeds at a significantly faster rate than that observed with either substrate alone. This indicates that the gluconeogenic flux was not saturated with each individual substrate in the previous conditions. Despite favorable conditions (availability of glutamate + pyruvate), no significant stimulation of alanine synthesis is observed. When VPA is added, the extraction of lactate diminishes whereas that of glutamine increases as described previously. Glucose production is again reduced at higher VPA concentrations. The total apparent oxidation of lactate plus glutamine is thus only modestly influenced by the addition of VPA.

Oxygen consumption by cortical tubules (Table IV). In parallel experiments, the oxygen consumption of cortical tubules was measured using as substrate either 1 or 5 m*M* glutamine or lactate with or without the highest concentration (10 m*M*) of valproate. It can be seen that the addition of each substrate stimulated the oxygen consumption over that observed in the absence of substrate and that this phenomenon is more marked with 5 than 1 m*M*. The addition of valproate decreased the respiration by 35–40% in all circumstances.

Discussion

This study describes the effects of VPA on the renal metabolism of fasted rats. VPA reduces the net uptake

Table III. Effect of Valproate on the Metabolism of Lactate + Glutamine by Renal Cortical Tubules of Fasted Rats[a]

	VPA			
	0	0.1 m*M*	1 m*M*	10 m*M*
1 m*M* lactate/0.1 m*M* pyruvate 1 m*M* glutamine/0.1 m*M* glutamate				
Lactate	−123.3 ± 9.5	−100.2 ± 11.8	−62.5 ± 6.4*	−40.1 ± 8.5*
Pyruvate	−5.0 ± 2.3	−1.2 ± 1.7	6.4 ± 3.0*	8.0 ± 2.4*
Glutamine	−59.7 ± 7.2	−69.1 ± 5.3	−93.0 ± 5.1*	−96.2 ± 6.3*
Glutamate	18.7 ± 5.3	15.0 ± 5.2	18.2 ± 5.4	16.6 ± 4.8
Malate	0.2 ± 0.3	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.3
Glucose	41.8 ± 3.7	39.8 ± 4.6	23.5 ± 2.2*	23.1 ± 2.4*
Citrate	−1.7 ± 1.2	−0.6 ± 0.6	−2.6 ± 1.2	−2.1 ± 1.5
Aspartate	−0.2 ± 1.6	−1.3 ± 0.5	−1.3 ± 0.7	−1.0 ± 1.5
Alanine	5.7 ± 1.2	5.2 ± 0.1	6.2 ± 1.3	5.7 ± 0.7
Ammonia	108.3 ± 8.2	137.6 ± 11.1	174.6 ± 7.8*	183.3 ± 12.7*
Valproate	NM	NM	NM	−999 ± 101
Metabolic rates				
To glucose	83.6 (44%)	79.6 (47%)	47.0 (30%)	46.2 (34%)
To CO_2 + unmeasured metabolites	87.4 (46%)	77.4 (45%)	87.6 (59%)	68.6 (53%)
5 m*M* lactate/0.5 m*M* pyruvate 5 m*M* glutamine/0.5 m*M* glutamate				
Lactate	−250.2 ± 10.9	−175.2 ± 19.6*	−132.5 ± 6.3*	−93.3 ± 5.3*
Pyruvate	−36.9 ± 1.3	−26.2 ± 1.9*	−13.3 ± 1.6*	−12.9 ± 1.5*
Glutamine	−128.2 ± 8.5	−153.9 ± 9.3	−183.3 ± 9.5	−188.4 ± 10.8*
Glutamate	52.4 ± 4.8	63.1 ± 4.2	69.9 ± 4.9	67.5 ± 4.0
Malate	1.6 ± 0.3	1.0 ± 0.3	1.3 ± 0.2	0.6 ± 0.2
Glucose	62.1 ± 5.5	49.8 ± 4.7	31.7 ± 3.5*	29.9 ± 3.1*
Citrate	−0.7 ± 1.2	−0.1 ± 0.5	−1.4 ± 0.9	−1.9 ± 0.8
Aspartate	6.0 ± 0.8	7.6 ± 0.4	5.0 ± 0.9	5.0 ± 0.6
Alanine	6.5 ± 0.3	7.3 ± 0.1	8.3 ± 0.7	6.7 ± 0.4
Ammonia	142.7 ± 8.1	209.5 ± 10.2*	250.1 ± 16.1*	265.6 ± 13.9*
Valproate	NM	NM	NM	−877 ± 101
Metabolic rates				
To glucose	124.2 (30%)	99.6 (28%)	63.4 (19%)	59.8 (20%)
To CO_2 + unmeasured metabolites	231.8 (56%)	184.1 (52%)	190.9 (58%)	163.6 (56%)

[a] Data refer to net production (+) or utilization (−) of metabolites in μmol/g wet wt^{-1} hr^{-1}. Mean ± SEM (six experiments). NM, not measured. *$P < 0.01$ control vs VPA. Analysis of variance for repeated measurements (performed on measured data only). The maximal oxidation, estimated from the carbon balance (omitting alanine) are presented along with the percentage of distribution of the lactate + glutamine metabolized toward gluconeogenesis and apparent oxidation.

Table IV. Effect of Valproate on Oxygen Consumption by Renal Cortical Tubules of Fasted Rats[a]

	No substrate	Substrate			
		1 m*M*		5 m*M*	
		Glutamine (*n*)	Lactate (*n*)	Glutamine (*n*)	Lactate (*n*)
Control	98.1 ± 18.8	134 ± 15	152 ± 22	225 ± 17	218 ± 25
Valproate (10 n*M*)	59.5 ± 12.2*	92 ± 2*	92 ± 11*	147 ± 21*	139 ± 29*

[a] Values are means ± SEM (*n*, number of experiments). Results are expressed as μmoles of oxygen consumed per gram tissue wet weight per 60 min.
* Significant difference from value obtained in the absence of valproate in the oxymeter.

of lactate by cortical tubules. VPA was demonstrated to inhibit significantly the activity of the sodium-dependent lactate transporter in brush border membrane vesicles of dog proximal tubules (K_i = 0.1 m*M*) (26). No effect was reported on the basolateral, sodium-independent, lactate transport however. If this occurs also in the rat and if the former structure contributes significantly to the overall uptake of lactate by cortical tubules (a preparation with open lumen), a reduction of lactate uptake is thus expected. This may in part explain both the reduced oxidation of and gluconeogenesis from lactate or pyruvate. It is possible that VPA also interferes with the mitochondrial entry of pyruvate within the renal tubules since this effect was described for brain mitochondria (28). Finally, VPA may also interfere more directly with both oxidation (by PDH inhibition) (29) and gluconeogenesis (by pyruvate carboxylase inhibition (12, 30)) from pyruvate.

Since valproate is itself metabolized, as suggested by the disappearance of this unnatural fatty acid from the flasks, valproyl-CoA and various related metabolites may be simultaneously produced (10). These metabolites may in turn influence the metabolism of lactate/pyruvate in various ways. First, a phosphorylation of PDH may contribute to its physiologic inhibition. Second, a sequestration of CoA (as valproyl-CoA) may also reduce the pyruvate dehydrogenase activity and therefore interfere with pyruvate oxidation. Similarly, a drop in acetyl-CoA, an obligatory activator of the pyruvate carboxylase enzyme (31) initiating glucose synthesis from pyruvate, could simultaneously occur and reduce gluconeogenesis. Both these mechanisms were described in rat hepatocytes (8, 11, 12) and were demonstrated in dog cortical renal tubules (17). In accord with this scheme, the finding of persistent glucose synthesis from glutamine suggests that the flux from pyruvate to oxaloacetate is affected by VPA more than the flux from oxaloacetate to glucose.

While suppressing lactate oxidation, valproate is apparently unable to produce NADH and to sustain respiration to the same extent observed in absence of VPA, since a significant reduction of respiration is observed. VPA also suppressed the small synthesis of glutamate and glutamine observed in the presence of lactate. This may be related to oxidative redox change (for glutamate synthesis) with or without a fall in tissue ATP (for glutamine synthesis). These changes were indeed observed *in vivo* in the dog kidney (26).

In the presence of glutamine, valproate increases glutamine extraction and provokes an accumulation of metabolites produced from glutamine such as glutamate, pyruvate, and lactate. This effect contrasts with the observation described above with lactate and may be related to a defect of pyruvate oxidation, occurring in the absence of significant interference with glutamine transport and partial degradation into tubular cells. The relative cellular oxidation induced by the slow valproate metabolism would be compensated by a faster flux of glutamine metabolism. Indeed, during the transformation of glutamine to oxaloacetate, 9-NADH are produced without complete oxidation of glutamine to CO_2. This partial metabolic flux may therefore increase in a compensatory fashion if the NADH production in the first part of the Krebs cycle is impaired by VPA. This process would lead to pyruvate accumulation, since the net oxidation of pyruvate is reduced by VPA. The accumulation of lactate and pyruvate induced by VPA is probably not due to the 50% inhibition of gluconeogenesis observed per se since a complete inhibition of gluconeogenesis by mercoptopicolinate does not duplicate this finding (32).

That the flux between glutamine and oxaloacetate is actually increased by VPA is also directly suggested by the fact that, at low concentration, VPA increases only modestly glucose production from glutamine (but not from lactate). A similar, but more marked stimulation was reported in the study of Martin *et al.* (16) and was also observed with dog tubules (17). The increased α-ketoglutarate dehydrogenase and PEPCK activity brought about in the rat by starvation may explain that this effect is less marked in fasted than in fed rats or in dogs (no PEPCK adaptation). Since the maximal gluconeogenic flux is not obtained when glutamine alone is used as the sole exogenous substrate (at 1 or 5 m*M*) (as compared with values measured with lactate + glutamine), an increment in glucose synthesis probably reflects an increased generation of oxaloacetate/malate.

It is surprising however that in these experiments, a large fraction of the carbon skeleton of glutamine remains unaccounted for. This may be explained by the production of large amounts of unmeasured metabolites produced from glutamine (α-ketoglutarate, succinate, succinyl-CoA, oxaloacetate, etc.) in the flask. Unfortunately, we have not measured all of the possible intermediates in the incubation fluid. In a previous study by Martin *et al.* (16), which was comparable to our study in many respects, no significant accumulation of α-ketoglutarate, fumarate, or glycogen was observed. Thus, the nature of these putative organic acids remains to be elucidated. If an inhibition of mitochondrial transport of pyruvate by VPA plays a significant role in the depressed pyruvate oxidation, it is possible that the direct intramitochondrial generation of pyruvate from glutamine (through malic enzyme, since PEPCK is a cytosolic enzyme in the rat kidney (32)) may help to maintain glutamine oxidation.

The fact that alanine synthesis remains relatively unaffected by either the substrates provided to the tubules and/or by VPA administration should be commented on. In that rat kidney, the alanine aminotransferase activity is very low (33). The modest alanine accumulation observed in all conditions suggests that this amino acid simply follows a proteolytic flux occurring with this preparation.

It should be pointed that our results observed with kidney tubules of fasted rats are closely related to those obtained by Martin *et al.* using a similar preparation in fed rats (16). In these experiments performed with 5 m*M* glutamine and various concentration of valproate (10^{-6} to 10^{-2} *M*), most of the observations made in our study were also found. However, $^{14}CO_2$ production from [1-^{14}C]glutamine was also measured and used as an indirect index of glutamine oxidation (flux through the α-ketoglutarate dehydrogenase). This parameter was not influenced by valproate. Nevertheless, when a carbon balance is calculated from the data of Martin *et al.* (16), a maximal apparent oxidation of 291.5 μmol/g dry wt$^{-1}\cdot$hr^{-1} (73 μmol/g wet wt$^{-1}\cdot$hr^{-1}) is found, which increases to 376 μmol/g wet wt (94 μmol/g dry wt) with increasing concentration of valproate, in a manner comparable to that observed in our study. Since decarboxylation of the α-ketoglutarate originating from glutamine should have released the $^{14}CO_2$, these isotopic data did not substantiate an increased flux between glutamine and oxaloacetate and stand in contradiction with the calculation of a mass transfer between glutamine and lactate/pyruvate plus unidentified metabolic intermediates. This discrepancy might be resolved if (i) the specific activity of glutamine changes during the incubation and (ii) a significant accumulation of metabolites such as GABA occurs.

However, and in contrast to our data, no significant accumulation of pyruvate, lactate, and aspartate or other intermediates of the tricarboxylic cycle was observed in these experiments performed in fed rats (16). It remains possible that VPA may exert a smaller metabolic effect in fed than in fasted animals due to the competition for metabolic oxidation of VPA with natural endogenous fatty acids. Indeed, the presence of exogenous substrate is able to decrease the oxidation of this drug.

In any case, our study in the rat confirms the findings in the dog (17) and demonstrates that VPA is able to increase the renal ammonia production by kidney tubules *in vitro*. Thus, the kidney may contribute to create an hyperammonemic state in rat as in man (34). It should be appreciated however that VPA increased the production of ammonia by less than 2-fold in all circumstances studied. This effect is modest and smaller than that induced by a physiologic stimulus such as metabolic acidosis (22). It is therefore probable that the renal effect of VPA alone may not explain per se the occurrence of hyperammonemic episodes. A concomitant decrement of ammonia detoxification by the liver (8, 9) most probably coexists to produce this complication of VPA therapy.

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