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Inhibitory Effect of Valproate on Weak Organic Acid Uptake in Rat Renal Proximal Tubules

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ABSTRACT. The effects of an antiepileptic drug, valproic acid (VPA), on transport mechanisms involved in renal excretion of anionic xenobiotics were investigated on rat renal proximal tubules *in vitro*. It was found that VPA (0.1–1 mM) dose dependently inhibited the baseline uptake of a marker organic anion, fluorescein, in the tubules. The inhibition could not be exclusively accounted for by competition between VPA and fluorescein. Taking into account a proposed relationship between the weak organic anion uptake and ammoniagenesis, the influence of VPA (0.5 mM) on the effects of glutamine and glutamate (both at 5 mM) on fluorescein uptake and ammonia production were examined. Glutamine stimulated ammonia production by the tubules, with the glutamine-induced ammoniagenesis being further augmented by VPA, while glutamate failed to affect the basal ammoniagenesis. Both glutamine (5 mM) and glutamate (5 mM) slightly inhibited fluorescein uptake, with the inhibitory effects not modified by VPA. Thus, there was no coincidence in the effects of VPA on organic anion uptake and renal ammoniagenesis. At the same time, the inhibitory effect of VPA (0.5 mM) on fluorescein uptake was largely overcome by addition of pyruvate (5 mM) to the incubation medium. In addition, VPA strongly inhibited glucose production from pyruvate. A known modulator of pyruvate metabolism, dichloroacetic acid (DCA, 1 mM), also inhibited fluorescein uptake, although its inhibitory effect was less pronounced than that of VPA. Both inhibitors failed to alter the tissue content of α-ketoglutarate or lactate but did slightly augment the pyruvate level. The inhibitory effects of VPA and DCA on the baseline fluorescein uptake were not additive, suggesting their similar intracellular targeting. It is assumed that the inhibitory effect of VPA on baseline fluorescein uptake in rat renal proximal tubules *in vitro* may be associated with its action on pyruvate metabolism. BIOCHEM PHARMACOL **58**;8:1371–1377, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. valproate; weak organic acid uptake; ammoniagenesis; gluconeogenesis; proximal tubules; rat kidney

The transport system underlying renal excretion of exogenous weak organic anions in RPTs† handles a great variety of drugs and environmental chemicals [1]. The first step in the renal elimination of xenobiotics is their carrier-mediated entry into RPT cells [2], which opens the way to the development of the tissue-specific side effects of some drugs. It has been shown that VPA, widely used as an antiepileptic drug, exerts various effects on renal functions *in vivo* by modulating both transport and metabolic properties of renal tubules [3–5]. Thus, the primary objective of this study was to ascertain whether VPA could compete with prototypic weak organic anions, such as PAH and fluorescein, for entry into RPT cells. A secondary objective was to test if the known effects of VPA on metabolism of RPT cells could be properly used for elucidating mechanisms of metabolic regulation of the transport system under study. At present, it is generally accepted that organic anions are transported across the basolateral membrane of RPT cells in exchange for cytoplasmic KG, an outward gradient of which is maintained owing to intracellular production of KG in concert with its Li^+-inhibitable, Na^+-coupled reuptake from the extracellular space [2]. Based on the model, weak organic acid uptake can be stimulated in two major ways: (1) by increasing Na^+ gradient across the basolateral membrane because of accelerated ATP production and/or (2) by elevating the cytoplasmic KG level due to its intracellular overproduction or/and its redistribution among extracellular, intramitochondrial, and cytoplasmic compartments.

Recently, we have shown [6, 7] that cadmium ions, as well as pent-4-enoate or maleate significantly increased the basal KG level and stimulated baseline fluorescein uptake in rat RPTs in an Na-dependent manner. The stimulation of fluorescein uptake was blocked by lithium ions, suggesting that the stimulatory effect of the above substances was dependent on the re-uptake of KG. In parallel, these agents increased the basal ammonia level but inhibited the glucose production from pyruvate. Inasmuch as the glutamate

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† *Abbreviations:* VPA, valproic acid; RPT, renal proximal tubules; PAH, *p*-aminohippurate; KG, α-ketoglutarate; PCA, dichloroacetic acid; and T/M, tubule/medium concentration ratio.

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dehydrogenase (L-glutamate: $NAD(P)^+$ oxidoreductase [deaminating]; EC 1.4.1.3) reaction is an essential step in both ammonia and KG production, it seemed likely that agents affecting ammoniagenesis would also influence KG metabolism, thus modifying the weak organic anion transport in RPTs. Since it was known [8–12] that VPA stimulated ammonia production in rat, dog, and human RPTs, in the present work we compared the effects of VPA on fluorescein uptake and ammonia production in rat RPTs in an attempt to elucidate the proposed interrelation between weak organic anion uptake and ammoniagenesis.

MATERIALS AND METHODS

Preparation of rat renal cortical slices and renal cortex fragment suspension, as well as methods of measurement of fluorescein uptake, glucose production, and metabolite levels, have been described previously [13]. In brief, the work was carried out on kidneys of male Wistar rats from a local source weighting 200–250 g. Rats were quickly decapitated and their kidneys removed and decapsulated. The outermost slices (0.5–0.8 mm) were prepared free-hand using a razor blade, with the renal surface being left intact. Slices were preincubated at 20° for 60 min in aerated substrate-free physiological buffer containing (in mmol/L): NaCl 104.7; KCl 15.3; $CaCl_2$ 1.5; $MgCl_2$ 2.5; $NaHCO_3$ 3.6; NaH_2PO_4 3.3; Na_2HPO_4 4.8; pH 7.1–7.3 (the standard solution). In some experiments, cold preincubation of slices was used to load the cells with VPA or PAH before they were incubated with fluorescein. In this case, slices were kept at 2–4° for 120 min without aeration in 10 mL of the standard physiological solution supplemented with 0.5 mM PAH or VPA. After preincubation, the slices were rinsed in the standard buffer at 20° for 1 min. For fluorescein uptake measurements, the slices were incubated at 20° in 10 mL of fresh aerated medium of the same composition plus fluorescein (the final concentration of fluorescein in the bath medium was 0.05 mM, if not specially indicated). Substances under test were added to bath medium from stock water solutions. In some experiments, the incubation medium of a low Na^+ content was used, containing (in mmol/L): NaCl 10; cholinechloride 110; $CaCl_2$ 1.5; $MgCl_2$ 2.5; $KHCO_3$ 3.6; K_2HPO_4 4.8 KH_2PO_4 3.3; pH 7.0–7.2.

After incubation, the slices were washed out in the standard buffer and the concentration of fluorescein accumulated in the superficial convoluted RPTs located at a constant depth (approx. 40 μm) from the renal surface was measured using a laboratory-built microfluorimeter with contact objective lens. These tubules, as characterized by polarization epimicroscopy, can be regarded as the proximal convoluted tubules (S_1 segments). The lumen of the tubules was open. The effects of various substrates and inhibitors at concentrations habitually used for studying renal transport and metabolism *in vitro* were for the most part examined at 20 min of incubation, i.e. on the rising phase of the uptake curve. Fluorescence intensity was measured in 40 different RPTs on the surface of each slice, whereas the background fluorescence was subtracted. Measurements were repeated on slices from 3 animals, so each point represents the mean of 120 individual records. Results are expressed as normalized concentration ratios, T/M, where the unit is a T/M value for equilibrium fluorescein uptake after the slices were incubated at 2–4° for 90 min. Uptake data are presented as means ± 2SE, which practically coincides with 95% confidence limits at N = 120. Thus, a difference between two means concerning uptake data may be considered significant with $P < 0.05$ when the confidence limits do not overlap each other.

Kinetic parameters K_m and V_{max} were calculated from the Lineweaver–Burk equation $1/v = K_m/V_{max}S + 1/V_{max}$, where S is substrate (fluorescein) concentration, by the method of the least squares with statistical weighing, as described previously [14]. The V_{max} values are presented in arbitrary units as means ± SD (the rate of fluorescein uptake was measured at eight fluorescein concentrations in the range from 30 to 200 μM). The renal cortex fragment suspension (final content 4–8 mg protein/mL), prepared without treating the tissue with proteases, was incubated at 20° for 30 min in flasks containing 0.5 or 1 mL of the standard buffer in a shaker at 70 cycles/min. All substances under test were added to the incubation medium from stock water solutions. Incubation was stopped by adding ice-cold 30% CCl_3COOH. After neutralization and centrifugation, glucose content in clear extracts was measured by the glucose oxidase (β-D-glucose: oxygen oxidoreductase; EC 1.1.3.4) technique using the Sigma kit (Sigma Chemical Co.). Contents of ammonia and KG were measured in the extracts by standard enzymatic methods [15]. Protein content in the pellets was measured by a modified Lowry method [16]. Metabolite levels are expressed as means ± SE, with the number of parallel probes (N) indicated in parentheses. The statistical significance of differences among groups in each experiment was estimated by using one-way analysis of variance followed by unpaired *t*-test.

Chemicals

Fluorescein (disodium salt, uranin) was obtained from Koch-Light Laboratories Ltd.; valproate, dichloroacetate, and α-ketoglutarate from Sigma; lactate, pyruvate, and succinate from Serva. Enzymes for metabolite determinations were obtained from Boehringer. Other reagents were of commercial grade.

RESULTS

As follows from the introduction, we could expect that VPA would either inhibit fluorescein uptake in the rat RPTs due to competition with the dye for common transport carriers or stimulate it owing to an elevation in the cytoplasmic KG level. It turned out that VPA (0.1–1 mM) significantly inhibited the baseline fluorescein uptake in a dose-dependent manner, regardless of the presence of Na^+

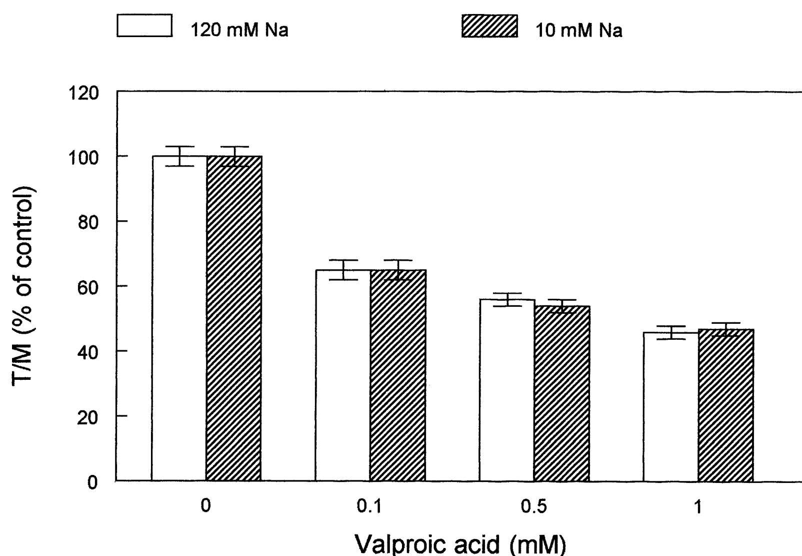

FIG. 1. Dependence of fluorescein uptake on VPA concentration in the incubation medium. Slices were preincubated in the substrate-free standard buffer at 20° for 60 min and then incubated with 0.05 mM fluorescein at 20° for 20 min in the medium at an Na^+ concentration of 120 or 10 mM. Bars represent the ratio of fluorescein concentrations in tubules and medium (T/M ratio) as a percentage of appropriate controls (fluorescein uptake in the VPA-free media). The absolute T/M values for the 120 and 10 mM Na^+ media averaged 2.71 ± 0.06 and 3.11 ± 0.04, respectively. Each bar is the mean of 120 individual measurements of RPTs on slices from 3 rats. Vertical lines show 95% confidence limits. Two means may be considered as significantly different ($P < 0.05$) if their confidence limits do not overlap each other.

in the bath medium (Fig. 1). Thus, it was of interest to determine the cause of the inhibitory effect of VPA on the weak organic anion uptake. In further experiments, bath medium with the standard (120 mM) Na^+ concentration was used to incubate renal slices or renal cortex fragment suspension. VPA was tested at a concentration of 0.5 mM, which was known [17] to be close to its therapeutic plasma concentration. At this concentration, VPA inhibited baseline fluorescein uptake by 45–50%, with the inhibitory effect on the uptake not increasing significantly in the course of incubation of the slices with fluorescein (inhibition by 54% and 48% after incubation for 40 min and 10 min, respectively) (data not shown).

In order to classify the type of inhibitory action of VPA, we evaluated its effect on the initial rate of uptake at various concentrations of fluorescein in the bath medium. As follows from the plot of the Lineweaver–Burk transformation (Fig. 2), both parameters of the Michaelis–Menten equation were influenced by VPA. It was calculated that V_{max} diminished from 70 ± 4 to 61 ± 1 arbitrary units ($P < 0.05$), while the apparent K_m increased from 0.18 mM (within the 95% confidence limits of 0.16 ÷ 0.21 mM) to 0.38 mM (within the 95% confidence limits of 0.37 ÷ 0.39 mM), i.e. the V_{max} value was reduced by only 13% whereas the K_m value doubled in the presence of 0.5 mM VPA in the bath medium. Hence, the inhibition appeared to be a of a competitive type. It seemed quite probable that valproate, itself being a weak organic anion, could compete with fluorescein for transport carriers at the basolateral membrane, thus contributing to the inhibition of fluorescein uptake in the rat RPTs. To verify this possibility, we performed cold preincubation of the slices with VPA (see Methods) to load the cells with the drug prior to incubation with fluorescein. Seeing that the incubation medium was nominally VPA-free, competition between fluorescein and VPA for entry into the RPT cells was unlikely. In parallel experiments, the cells were preloaded with PAH, a typical competitive inhibitor of fluorescein uptake in RPTs. As

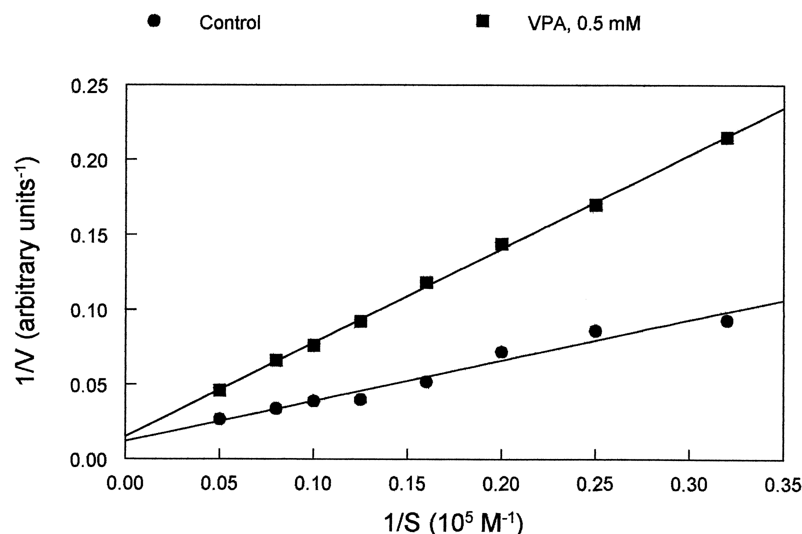

FIG. 2. Lineweaver–Burk plots for fluorescein uptake in the absence and presence of VPA. Abscissa: reversal concentration of fluorescein in the bath; ordinate: reversal rate of fluorescein uptake. After preincubation (20°, 60 min), the slices were incubated with fluorescein (8 concentrations in the range of 0.03–0.2 mM) at 20° for 10 min in the standard medium containing or not VPA (0.5 mM). The coefficient of correlation with the straight line was 0.97 for the control and 0.99 for 0.5 mM valproate.

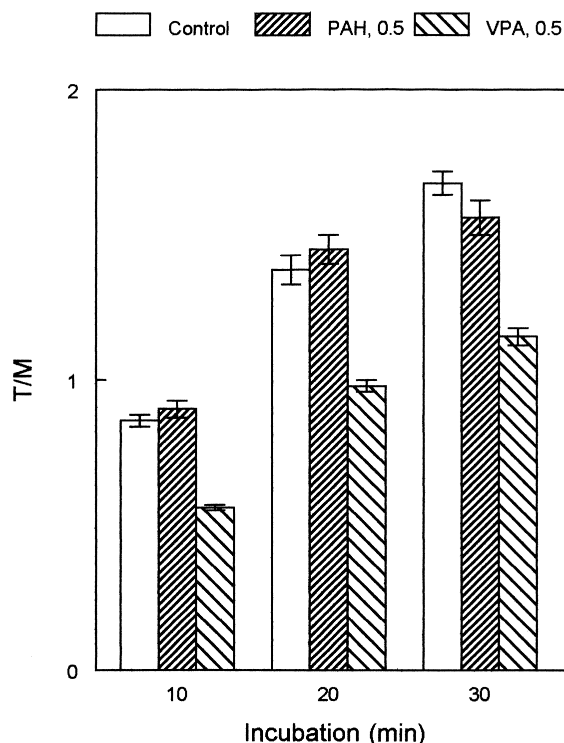

FIG. 3. Fluorescein uptake after cold preincubation with PAH or VPA. Before incubation in the standard medium with fluorescein (0.05 mM) at 20° for 20 min, the slices were preincubated in the standard buffer containing PAH or VPA (both at 0.5 mM) at 2–4° for 120 min. Designations as in the legend to Fig. 1.

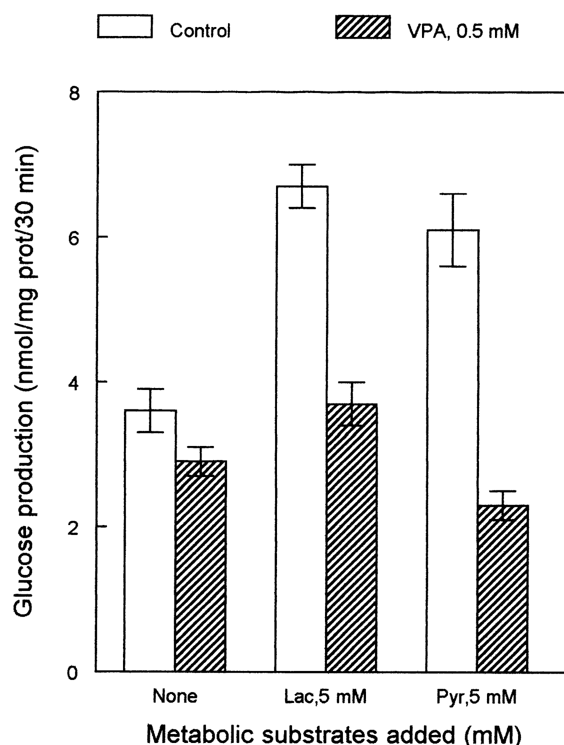

FIG. 4. Effect of VPA on glucose production from lactate (Lac), pyruvate (Pyr), or endogenous substrates by rat renal cortex fragment suspension. The suspension was incubated in the standard medium at 20° for 30 min. Bars show means ± SE (N = 8). Significance: the difference among groups, P < 0.0001 (F = 16.97); Lac + VPA vs Lac, *P* < 0.01; Pyr + VPA vs Pyr, *P* < 0.01; Lac + VPA vs Pyr + VPA, *P* < 0.05.

seen in Fig. 3, cold preincubation with PAH did not affect fluorescein uptake after the 20-min incubation, while a rather strong inhibitory effect of VPA (approximately 30% of inhibition) persisted under these conditions.

The above data suggested that the inhibitory effect of VPA on baseline fluorescein uptake in rat RPTs could not be entirely accounted for by competition for the transport sites at the basolateral membrane. This is why we further investigated if inhibition of fluorescein uptake could be mediated by an influence of the drug on the metabolism of RPT cells. As was to be expected, VPA (0.5 mM) influenced two important metabolic processes in the rat RPTs. First, it significantly inhibited glucose production from lactate and, especially, from pyruvate (by 45 and 62%, respectively), but not from endogenous substrates (Fig. 4). Second, VPA augmented ammonia production from 5 mM glutamine, but failed to affect the basal ammonia level in the RPT cells (Fig. 5). It was noteworthy that 5 mM glutamate, as contrasted to glutamine, did not increase ammonia production in the rat RPTs and that VPA was without effect on renal ammoniagenesis in the presence of glutamate in the incubation mixture. At the same time, VPA (0.5 mM) only slightly altered the basal intracellular content of KG in the renal cortex fragment suspension, with the KG content averaging 0.117 ± 0.005 (N = 11) nmol/mg protein after incubation for 30 min with VPA, as compared to 0.102 ± 0.007 (N = 16) nmol/mg protein in the appropriate control ($P > 0.2$).

In order to test the expected relationship between fluorescein uptake and substrate-induced ammoniagenesis, we investigated the effects of glutamine and glutamate on weak organic anion uptake. It was found that glutamine had a biphasic effect on fluorescein uptake: at 0.5 and 1 mM, the substrate stimulated fluorescein uptake in the rat RPTs by 11 and 27%, respectively, while at 2.5 mM and above, exogenous glutamine significantly inhibited uptake. Glutamate at a concentration of 0.5 mM was without effect, but at 1 mM and above its influence on fluorescein uptake was inhibitory as well (data not shown). As seen in Fig. 6, the inhibition of fluorescein uptake by 5 mM glutamine or 5 mM glutamate was not modulated by VPA, which lowered the weak organic anion uptake in the presence of either of the substrates to the same level as it did in the substrate-free bath medium. Thus, the changes in fluorescein uptake in the rat RPTs were not coincident with the alterations in the substrate-induced ammoniagenesis. As for the other metabolic substrates, KG, lactate, pyruvate, succinate, and acetate were capable of stimulating baseline fluorescein uptake in rat RPTs, but only KG (0.5 mM) and pyruvate (5 mM) succeeded in substantially overcoming the inhibitory effect of VPA on uptake (Fig. 7). Taking into account that

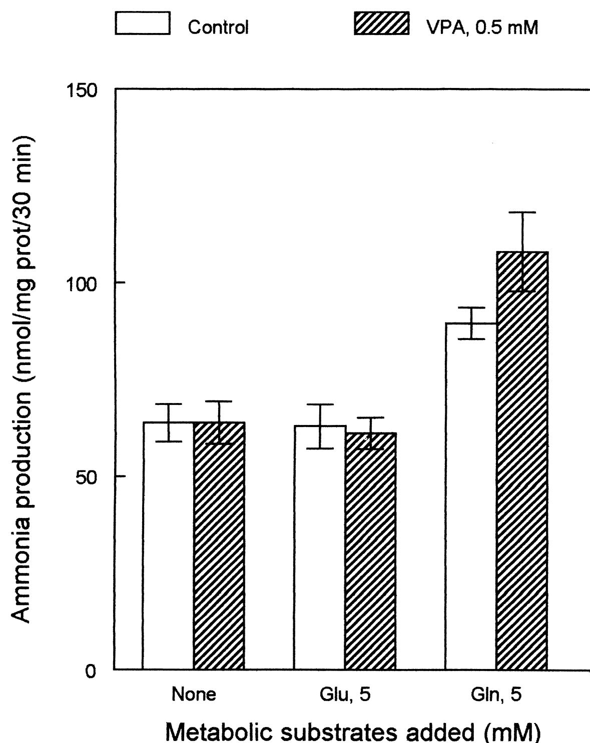

FIG. 5. Effect of VPA on ammonia production from glutamine (Gln), glutamate (Glu) and endogenous substrates by rat renal cortex fragment suspension. The suspension was incubated in the standard medium at 20° for 30 min. Bars show means ± SE (N = 8). Significance: the difference among groups, P < 0.0001 (F = 10.11); Gln vs control, *P* < 0.05; Gln vs Glt, *P* < 0.01; Gln + VPA vs Gln, *P* < 0.1.

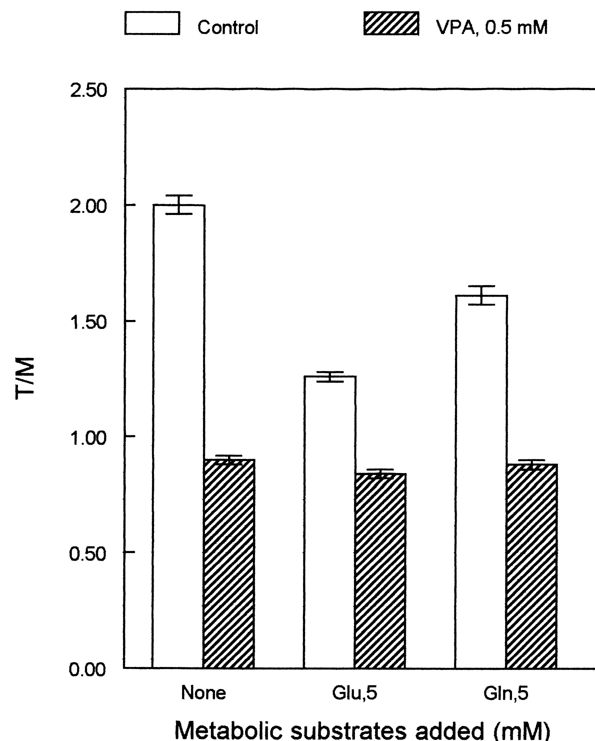

FIG. 6. Effect of VPA on fluorescein uptake in the presence of glutamine (Gln) or glutamate (Glu) in the incubation medium. Incubation in the standard medium. Other conditions and designations as in the legend to Fig. 1.

an influence of VPA on the metabolism of RPT cells might be associated with a disturbance in pyruvate metabolism [8, 10], we compared the effects of VPA with those of DCA, which has been supposed [18] to activate pyruvate dehydrogenase complex and to inhibit pyruvate carboxylase (pyruvate: CO_2 ligase [ADP-forming]; EC 6.4.1.1). Similarly to VPA, DCA (0.1–1 mM) dose dependently inhibited baseline fluorescein uptake in rat RPTs, with its effect being overcome by 5 mM pyruvate (not shown). The KG level in the rat RPT cells was not affected by DCA (1 mM): the KG content averaged 0.117 ± 0.013 (N = 10) nmol/mg protein in the presence of DCA, as compared to 0.102 ± 0.007 (N = 16) nmol/mg protein in the control ($P > 0.2$). Both VPA (0.5 mM) and DCA (1 mM) slightly augmented pyruvate content in rat RPT cells (from 0.615 ± 0.047 [N = 17] in the control to 0.816 ± 0.057 [N = 16; $P < 0.05$] and 0.824 ± 0.055 [N = 16; $P < 0.01$] nmol/mg protein in the presence of VPA and DCA, respectively), while the intracellular lactate content was not altered by the inhibitors (11.1 ± 0.7; 11.1 ± 1.3 and 10.9 ± 0.8 nmol/mg protein, respectively; N = 18). The inhibitory effects of VPA and DCA on baseline fluorescein uptake were not additive: the T/M values were equal to 2.18 ± 0.04, 0.92 ± 0.02, 1.68 ± 0.04, and 1.22 ± 0.01 in control and in the presence of VPA, DCA, and VPA + DCA, respectively. Thus, it appeared that DCA attenuated the inhibition of fluorescein uptake by VPA.

DISCUSSION

The data obtained in this study evidenced that VPA inhibited baseline fluorescein uptake in rat RPTs in a dose-dependent manner, regardless of the presence of Na^+ in the incubation medium. It might well be that VPA competed with fluorescein for common transport sites. A similar conclusion was drawn on the basis of data showing that PAH inhibited uptake of VPA in the rat brain [19]. Kinetically, inhibition by VPA (0.5 mM) of fluorescein uptake in rat RPTs seemed to be competitive: the K_m value increased more dramatically than the V_{max} value decreased. However, the inhibitory effect of VPA on fluorescein uptake could not be explained merely by competition for transport mechanism. When the RPT cells were preloaded with VPA in the course of cold preincubation, its effect on uptake persisted during subsequent incubation of the renal slices with fluorescein in the VPA-free medium, contrary to the effect of PAH, a typical competitive inhibitor of fluorescein transport. Hence, it was more probable that the inhibitory effect of VPA on fluorescein uptake was related to its action on the cellular metabolism.

In accordance with the data of other researchers [8, 10, 12], in our experiments VPA (0.5 mM) inhibited gluconeogenesis from lactate (and pyruvate), while increasing

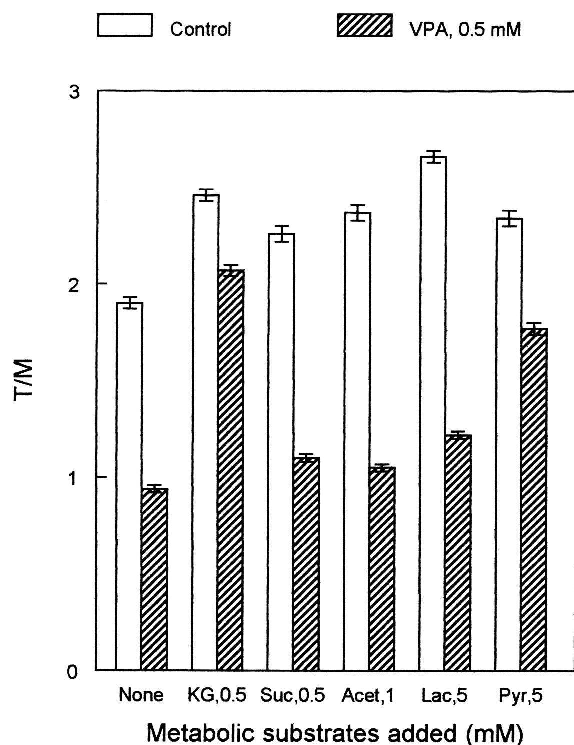

FIG. 7. Effect of VPA on fluorescein uptake in the presence of KG, succinate (Suc), acetate (Acet), lactate (Lac), or pyruvate (Pyr) in the incubation medium. Incubation in the standard medium. Other conditions and designations as in the legend to Fig.

ammonia production from glutamine (but not from glutamate). In contrast with our expectations, no coincidence was observed between changes in fluorescein uptake and substrate-induced ammonia production in rat RPTs, as affected by VPA. In the presence of glutamine (5 mM) in the incubation medium, ammonia production increased as compared to the control, while fluorescein uptake was significantly inhibited by external glutamine at this concentration. Glutamate (5 mM) also inhibited fluorescein uptake, but did not affect renal ammoniagenesis. When added together with glutamine (but not with glutamate), VPA further augmented ammonia production. At the same time, VPA failed to overcome the inhibitory effect of glutamine on fluorescein uptake. The above-mentioned data fall into line with the assumption [8, 9] that VPA stimulates renal ammonia production by activating the glutaminase (L-glutamine amidohydrolase; EC 3.5.1.2) reaction, while deamination of glutamate is not affected by the drug. It is evident from our data that activation of glutaminase is without effect on fluorescein uptake in rat RPTs. Thus, augmentation of basal ammonia production in rat RPTs by pent-4-enoate, maleate, or cadmium ions, which was accompanied by stimulation of baseline fluorescein uptake [6, 7], is not likely to be related to oxidation of endogenous glutamine. The failure of exogenous glutamate to stimulate fluorescein uptake raises the question as to why an acceleration of intramitochondrial KG production does not result in an increase in fluorescein uptake in rat RPTs.

External KG or pyruvate (but not acetate, lactate, and succinate) was able to largely overcome the inhibitory effect of VPA on fluorescein uptake. Recently, it has been shown in our laboratory that KG was capable of stimulating fluorescein uptake in rat RPTs pretreated with metabolic poisons [20]. Thus, it is very probable that the marked augmentation of fluorescein uptake by KG in the presence of VPA is mediated by the entry of the dicarboxylate into the cells with subsequent acceleration of its exchange for extracellular fluorescein across the basolateral membrane. As for pyruvate, it was proposed [2] that its stimulatory effect on renal uptake of weak organic anions such as PAH or fluorescein is mediated by metabolic transformations, resulting in an elevation of the intracellular KG level. In this context, it is of interest that VPA is thought to inhibit pyruvate metabolism in RPT cells [8, 10]. If so, the protective influence of exogenous pyruvate on the VPA effect on fluorescein uptake might be explained by the notion that an excess of pyruvate blocks the inhibitory action of VPA on pyruvate metabolism. Another agent known [18] to affect renal pyruvate metabolism, DCA, exerted a similar effect on fluorescein uptake in rat RPTs. Neither VPA nor DCA affected the intracellular contents of KG and lactate, but both agents slightly augmented the pyruvate content. Nevertheless, the inhibition of baseline fluorescein uptake by VPA might hardly be accounted for by the decrease in the cytoplasmic redox state in RPT cells (as estimated by the lactate/pyruvate ratio), since external lactate (5 mM), which induced the reductive shift in rat RPTs [13], failed to overcome the inhibitory action of VPA on the weak organic anion uptake.

The inhibitory effect of DCA (1 mM) on baseline fluorescein uptake in rat RPTs was moderate, as compared to that of 0.5 mM VPA. However, when the inhibitors were added together, fluorescein uptake was diminished to a lesser extent than in the presence of VPA alone, as if DCA attenuated the effect of VPA. It has been assumed that DCA inhibits the pyruvate carboxylase reaction, while at the same time activating the pyruvate dehydrogenase complex in rat RPTs [18], whereas VPA is likely to inhibit both reactions [8]. Previously, we have shown [13] that an inhibition of pyruvate carboxylase results in a marked diminution of baseline fluorescein uptake in rat RPTs. Thus, the inhibitory effects of VPA and DCA on the uptake could well be conditioned by their influence on the pyruvate carboxylase reaction. Then, the attenuation of the effect of VPA on fluorescein uptake by DCA suggests that the inhibitory effect of VPA is associated with its inhibitory action at both the pyruvate carboxylase and pyruvate dehydrogenase stages. At the same time, the failure of acetate to overcome the inhibitory effect of VPA on fluorescein uptake evidences that the proposed inhibition of pyruvate carboxylase by VPA is not due to a decrease in the acetyl-CoA content, which is known [21] to be a powerful activator of the enzyme.

In conclusion, it seems evident that the inhibitory effect of VPA on renal weak organic anion uptake *in vitro* does

not result exclusively from its direct action on the transport mechanism, but, rather, is mediated in great part by an influence of the drug on cellular metabolism. The inhibition of the uptake by VPA is not likely associated with alterations in substrate-induced ammoniagenesis and, as a consequence, in KG metabolism. It is more probable that some disturbance in pyruvate metabolism is responsible for the effect of VPA on the transport process under consideration. Further elucidation of reasons for the inhibitory effect of VPA may throw light on the mechanisms of metabolic regulation of weak organic anion transport in RPTs.

This work was presented at the XXXIII International Congress of Physiological Sciences (St. Petersburg, Russia, 1997) and published in abstract form [22].

References

1. Moeller JV and Sheikh MI, Renal organic anion transport system: pharmacological physiological and biochemical aspects. *Pharmacol Rev* **34:** 315–358, 1983.
2. Pritchard JB and Miller DS, Proximal tubular transport of organic anions and cations. In: *The Kidney: Physiology and Pathophysiology* (Eds. Seldin DW and Giebisch G), pp. 2921–2945, Raven Press, Ltd., New York, 1992.
3. Warter JM, Brandt C, Marescaux C, Rumbach L, Micheletti G, Chabrier G, Krieger J and Imler M, The renal origin of sodium valproate-induced hyperammonemia in fasting humans. *Neurology* **33:** 1136–1140, 1983.
4. Fisher JE, Nau H and Lasher W, Alterations in the renal excretion of valproate and its metabolites after chronic treatment. *Epilepsia* **32:** 146–150, 1991.
5. Korinthenberg R, Wehrle L and Zimmerhackl LB, Renal tubular disfunction following treatment with anti-epileptic drugs. *Eur J Pediatr* **153:** 855–858, 1994.
6. Nikiforov AA and Ostretsova IB, Stimulation of weak organic acid uptake in rat renal tubules by cadmium and nystatin. *Biochem Pharmacol* **47:** 815–820, 1994.
7. Ostretsova IB and Nikiforov AA, Weak organic acid uptake in rat renal tubules in vitro: Stimulation by pent-4-enoic acid. *Comp Biochem Physiol* **117C:** 1–6, 1997.
8. Gougoux A and Vinay P, Metabolic effects of valproate on dog renal cortical tubules. *Can J Physiol Pharmacol* **67:** 88–97, 1988.
9. Martin G, Michoudet C and Baverel G, Stimulation of glutamine metabolism by the antiepileptic drug, sodium valproate, in isolated dog kidney tubules. *Biochem Pharmacol* **38:** 3947–3952, 1989.
10. Doval M, Culebras M, Lopez-Farre A, Rengel M, Gougoux A, Vinay P and Lopez-Novoa JM, Effect of valproate on lactate and glutamine metabolism by rat renal cortical tubules. *Proc Soc Exp Biol Med* **190:** 357–365, 1989.
11. Rumbach L, Cremel G, Marescaux C, Warter JM and Waksman A, Valproate-induced hyperammonemia of renal origin. Effects of valproate on glutamine transport in rat kidney mitochondria. *Biochem Pharmacol* **38:** 3963–3967, 1989.
12. Martin G, Durozard D, Besson J and Baverel G, Effect of the antiepileptic drug sodium valproate on glutamine and glutamate metabolism in isolated human kidney tubules. *Biochim Biophys Acta* **1033:** 261–266, 1990.
13. Nikiforov AA and Ostretsova IB, Effects of inhibitors of gluconeogenesis on weak organic acid uptake in rat renal tubules. *Biochem Pharmacol* **44:** 2213–2221, 1992.
14. Bresler VM and Nikiforov AA, Double dependence of organic acid active transport in proximal tubules of surviving frog kidney on sodium ions. *Biochim Biophys Acta* **468:** 81–99, 1977.
15. Bergmeyer HU (Ed) *Methods of Enzymatic Analysis*. New York and London, Verlag Chemie Weinham, 1974.
16. Markwell MAK, Haas SM, Beiler LL and Tolbert ME, A modification of Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* **87:** 206–210, 1978.
17. Chadwick DW, Concentration–effect relationships of valproic acid. *Clin Pharmacokinet* **10:** 155–163, 1985.
18. Lacey JH and Randle PJ, Inhibition of lactate gluconeogenesis in rat kidney by dichloroacetate. *Biochem J* **170:** 551–560, 1978.
19. Adkison KD and Shen DD, Uptake of valproic acid into rat brain is mediated by a medium-chain fatty acid transporter. *J Pharmacol Exp Ther* **276:** 1189–1200, 1996.
20. Nikiforov AA and Ostretsova IB, Effects of metabolic substrates and inhibitors on weak organic anion transport in rat renal proximal tubules. *Fiziol Zh Im I M Sechenova* **83:** 178–189, 1997.
21. Wirthensohn G and Guder WG, Renal substrate metabolism. *Physiol Rev* **66:** 469–497, 1986.
22. Zagryadskaya EI, Ostretsova IB and Nikiforov AA, Effect of valproate on fluorescein uptake in rat renal tubules. In: *Proceedings of the XXXIII International Congress of Physiological Sciences*, St. Petersburg, Russia, 30 June–5 July, 1997.