Metabolite Anion Carriers Mediate the Uptake of the Anionic Drug Fluorescein in Renal Cortical Mitochondria

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ABSTRACT

The fluorescent organic anion fluorescein (FL) accumulates in proximal tubular cells of the kidney during renal secretion. In freshly isolated and permeabilized proximal tubular cells, the uptake was reduced but still sensitive to probenecid, suggesting a concentrative mechanism that is associated with intracellular compartments. Previous studies have shown that one of these compartments may be mitochondrial. In this study, we further investigated the transport characteristics of FL in isolated rat kidney cortex mitochondria. Mitochondrial uptake of 100 μM FL was rapid, with an initial rate of 60 pmol/mg protein-min, and reached equilibrium after 5 min. To characterize the transport system(s) involved, FL uptake was studied in the absence and presence of substrates or inhibitors specific for the various mitochondrial anion carriers. Phenylsuccinate (10 mM), an inhibitor of the α-ketoglutarate carrier, reduced uptake significantly with a maximum inhibition of 33% and an inhibitory constant (−log IC50) of 4.0 ± 0.4 (P < .05). The apparent Km for the phenylsuccinate-corrected FL uptake was 1.3 ± 0.3 mM with a Vmax of 260 ± 26 pmol/mg protein-15 s. Substrates for the tricarboxylate and glutamate-aspartate carriers significantly reduced the uptake of 100 μM FL with −log IC50 values of 4.6 ± 0.4 (citrate), 5.5 ± 0.3 (glutamate), and 4.1 ± 0.4 (aspartate). Substrates for the monocarboxylate and dicarboxylate carriers were without effect. The anionic drugs, valproate, indomethacin, and salicylate, significantly reduced FL uptake, whereas cephaloglycin and cephaloridine had no effect. Finally, a combination of phenylsuccinate, glutamate, and citrate reduced the uptake by 66%, indicating that at least three metabolite carriers contribute concomitantly to intramitochondrial FL transport.

Renal excretion is a major route of drug elimination from the body. Proximal tubular cells (PTC) possess separate carrier systems for the active secretion of organic anions and cations. As a result of an efficient uptake mechanism against the membrane potential, anionic drugs tend to accumulate within PTC, sometimes leading to nephrotoxicity (Pritchard and Miller, 1993; Ulrich, 1997; Roch Ramel, 1998). The organic anion transport system (OAT1) mediates the uptake of organic anions across the basolateral membrane via exchange with intracellular dicarboxylates (Sekine et al., 1997; Sweet et al., 1997). Among the wide range of substances accepted by OAT1, p-aminohippurate and fluorescein (FL) can be considered as excellent model substrates (Sullivan et al., 1990; Masereeuw et al., 1994; Welborn et al., 1998). The uptake of organic anions is driven by the intracellular dicarboxylate concentration, which is maintained by the transmembrane Na⁺ gradient. Dicarboxylates capable of exchanging with organic anions are adipate, suberate, glutarate, and α-ketoglutarate. α-Ketoglutarate is by far the most abundant potential dicarboxylate counterion within the PTC, and it has been shown that p-aminohippurate and FL uptake increases with increasing internal α-ketoglutarate concentration. The activity of the Na⁺/dicarboxylate exchanger accounts for approximately 60% of organic anion uptake (Welborn et al., 1998), whereas the remainder can be explained by intracellularly stored α-ketoglutarate (Pritchard, 1995).

Confocal microscopic images showed that the intracellular distribution of FL is not confined to the cytosol. At least two different compartments seem to be involved in the intracellular accumulation of this anion, viz., mitochondria and a vesicular compartment that has yet to be identified (Miller et al., 1993; Masereeuw et al., 1994; Miller and Pritchard, 1994). Mitochondrial uptake of FL in isolated PTC was susceptible to inhibition by phenylsuccinate, suggesting that an active process may be involved (Masereeuw et al., 1996). In the same study, respiration measurements and [14C]succinate uptake experiments showed a competitive interaction between FL and the uptake of the endogenous metabolite succinate in mitochondria.

Uptake of anionic compounds into mitochondria has to proceed against a negative membrane potential, and, therefore, must be either energy-dependent or linked to the transport of other chemicals. The mitochondrial uptake of the anionic drug FL could be mediated by one or more anion carrier proteins located in the inner membrane. So far, eight major anion carriers have been described (Fonyo et al., 1976;
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LaNoeu and Schoolwerth, 1979; Schoolwerth and LaNoeu, 1985; Gullans and Hebert, 1996). Because the adenine nucleotide translocase and the phosphate transporter have fairly narrow substrate specificities, it is unlikely that they mediate the uptake of FL. Each of the other six transporters could be involved in the uptake of the anionic dye, viz., the monocarboxylate, dicarboxylate, tricarboxylate, α-ketoglutarate, glutamate, and glutamate-aspartate transporters.

Several anionic drugs are known to cause respiratory toxicity in PTC. Part of the mechanism underlying this toxicity might be explained by mitochondrial uptake of these xenobiotics. Valproate, for example, is known to inhibit mitochondrial uptake of succinate and pyruvate (Rumbach et al., 1989), and the mitochondrial toxicity of equestin, an antibiotic, is thought to be caused by inhibition of substrate anion carriers (Konig et al., 1993). The cephalosporins, cephaloridine and cephaloglycin, are also known to reduce the uptake of, and respiration with, succinate in renal cortical mitochondria (Tune, 1993).

The objective of this study was to investigate further the uptake characteristics of the prototypal organic anion, FL, in renal cortical mitochondria. We used isolated rat PTC and kidney cortex mitochondria to demonstrate that mitochondrial FL uptake is mediated by the α-ketoglutarate, tricarboxylate, and glutamate-aspartate carriers.

Experimental Procedures

Materials. FL, succinate, phenylsuccinate, butylmalonate, and EGTA were purchased from Aldrich Chemie (Steinheim, Germany). BSA and HEPES were obtained from Boehringer Mannheim (Mannheim, Germany). Sodium salicylate was obtained from O.P.G. (Utrecht, the Netherlands). All other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany) or Sigma (St. Louis, MO).

Isolation of PTC and FL Uptake. Rat kidney PTC were isolated according to a method previously described by Maserereeuw et al. (1994). Purified PTC were suspended in incubation buffer containing 117.5 mM NaCl, 1.2 mM MgSO4, 0.95 mM KH2PO4, 22.5 mM NaHCO3, 11.1 mM glucose, and 2.5 mM CaCl2, adjusted to pH 7.4. Cell yield was 25 to 40 × 106 cells per ml and viability was higher than 90% as judged by trypan blue exclusion and lactate dehydrogenase release. Approximately 2 × 106 cells were permeabilized with saponin treatment (20 μg/ml) in 500 μl of carbogen-saturated incubation buffer at 37.5°C in a rotary shaker (220 rotations/min) for 15 min (van Os et al., 1988). The percentage of leaky cells was determined by trypan blue exclusion. Control cells were prestained for 15 min without saponin. The permeabilized cells as well as the control cells were incubated with different concentrations of FL for 3 min in the absence or presence of 5 mM probenecid. Incubations were stopped by the addition of 5 ml of ice-cold washing buffer containing 140 mM NaCl, 4 mM KCl, 1.2 mM MgSO4, 0.95 mM KH2PO4, 11.1 mM glucose, and 2.5 mM CaCl2, adjusted to pH 7.4. After rapid centrifugation, the cells were washed twice with washing buffer. All washing and centrifugation procedures were carried out at 4°C. The cells were lysed with 2.5 ml of distilled water and vortexed before analysis (Maserereeuw et al., 1994).

Isolation and Function of Kidney Cortex Mitochondria. Rat kidney cortex mitochondria were isolated by a modification (Maserereeuw et al., 1996) of the method described by Cain and Skilletler (1987). Briefly, male Wistar Hanover rats weighing 230 to 280 g were anesthetized i.p. with pentobarbital (60 mg/kg b.wt.). Heparin (125 U/100 g b.wt.) was administered in the femoral vein. The kidneys were perfused via the aorta with an ice-cold solution containing 140 mM NaCl and 10 mM KCl, after which the kidneys were removed. The following steps were all carried out at 4°C. The kidney cortex was homogenized by hand in ice-cold isolation buffer (300 mM mannitol, 10 mM HEPES, 1 mM EGTA, and 1 mg/ml BSA, pH 7.4) in a Potter-Elvehjem homogenizer with a Teflon pestle (Braun, Melsungen, Germany). The clearance between pestle and Potter tube was 0.5 mm. The homogenate was filtered through two layers of woven gauze. Mitochondria were isolated from the homogenate by differential centrifugation, and were finally resuspended in respiration buffer containing 210 mM mannitol, 10 mM KCl, 10 mM KH2PO4, 0.5 mM EGTA, and 60 mM Tris-HCl, pH 7.4, and kept on ice.

The purity of the mitochondrial fraction was determined by assaying the specific activity of certain marker enzymes as described by Russel et al. (1988), viz., succinate dehydrogenase for mitochondria, Na~−K~−ATPase for basolateral membranes, alkaline phosphatase for brush-border membranes, and acid phosphatase for lysosomes. Succinate dehydrogenase showed an enrichment of 3.2 ± 0.3-fold, Na~−K~−ATPase of 1.13 ± 0.2-fold, alkaline phosphatase of 1.3 ± 0.1-fold, and acid phosphatase of 1.4 ± 0.1-fold (n = 5).

Respiration experiments were performed to assess the functional integrity of the mitochondria. Oxygen consumption in mitochondrial suspensions was measured with a Clark-type platinum electrode using 1 mg of mitochondrial protein in respiration buffer at 30°C as previously described by Cain and Skilletler (1987). Energyization of the mitochondria was achieved by the addition of 10 mM succinate in the presence of 5 μM rotenone to block electron transport proximal to succinate entry into the citric acid cycle. Subsequently, ADP-stimulated respiration (state 3) was measured in the presence of 0.3 mM ADP. State 4 was determined after exhaustion of ADP. Respiratory control ratio (RCR) values (ratio of state 3/state 4) were calculated as an indication of functional integrity. For succinate-linked respiration, RCR values above 3 are indicative of intact and well coupled mitochondria (Cain and Skilletler, 1987). Freshly isolated mitochondria exhibited a RCR of 3.7 ± 0.1 (n = 4), and mitochondria that were preincubated at 10°C for 1 h exhibited a RCR of 3.1 ± 0.3 (n = 5). This difference is not significant, indicating that our mitochondrial preparation was of good quality and was tightly coupled, even after preincubation for 1 h.

Mitochondrial FL Uptake. Mitochondria were preincubated at 10°C for 1 h in respiration buffer in the presence or absence of a substrate or specific inhibitor of one of the mitochondrial anion carriers or anionic drug. The concentration of mannitol in the respiration buffer was adjusted to a final osmolality of 290 mM in the presence or absence of substrates and inhibitors. Incubation was started by the addition of 25 μl of mitochondrial suspension to 100 μl of respiration buffer supplemented with the desired concentration of FL with or without coadministration of the substrate or inhibitor. Uptake was stopped after the desired incubation period by the addition of 2.5 ml of ice-cold stop buffer containing 190 mM mannitol, 10 mM KCl, 0.5 mM KH2PO4, 0.5 mM EGTA, and 60 mM phenylsuccinate, pH 7.4. Mitochondria and extramitochondrial medium were quickly separated by using rapid vacuum filtration through Whatman GF/C filters (Omnilabo International, Breda, the Netherlands). Filters were washed twice, and 1 ml of 0.1 M NaOH was added, followed by thorough vortexing to extract the FL from the filter.

Biochemical Analysis. Fluorescence of the lysed cells was measured using a Shimadzu RF-5000 spectrofluorophotometer (Shimadzu Corporation, Kyoto, Japan). Because relative fluorescence is dependent on the amount of protein used, the concentrations were calculated by comparing the fluorescence intensities with a calibration curve of spiked samples of blank cell suspensions with different concentrations of FL as described previously (Maserereeuw et al., 1994). In mitochondrial uptake experiments, fluorescence in samples that were extracted from the Whatman GF/C filters was measured using a Perkin-Elmer LS-50 spectrofluorophotometer (Perkin-Elmer, Nieuwekerk aan de IJssel, the Netherlands). The protein concentration was determined using the Folin–Ciocalteu reagent.
content in each sample was determined by using the Bio-Rad protein assay (München, Germany) with BSA as the protein standard.

Data Analysis. All values are expressed as the mean ± S.E.; n is the number of different preparations studied. Statistical differences between means were first assessed by one-way analysis of variance, followed by Student's t test with Bonferroni correction. Differences were considered significant if P < .05. Nonlinear regression analysis was performed using GraphPad Prism 3.0 for Windows 95 (GraphPad Software Inc., San Diego, CA). In concentration-dependent FL uptake, the maximal rate of carrier-mediated transport, V_max (picomoles per milligram of protein·15 s), and the Michaelis-Menten constant, K_m (mM), were determined. In log-concentration inhibition analysis, curves were analyzed according to a one-site competition model. The IC_{50} values, the concentrations of the inhibitors causing 50% inhibition of FL uptake, were determined. The goodness of fit was determined by R^2.

Results

FL Uptake by PTC. Transport of FL into PTC is an active process because it occurred against the electrochemical gradient. Moreover, FL uptake was concentration-dependent and sensitive to inhibition by probenecid (Fig. 1). In permeabilized cells, the uptake of the dye was significantly decreased. Permeabilization was achieved by saponin treatment, which resulted in 90 ± 1% (n = 4) leaky cells as assessed by trypan blue exclusion. Probenecid further reduced FL uptake in these cells, indicating that the drug affects compartmentation. Because previous studies have shown that mitochondria may be one of the compartments involved, we studied the interaction between FL and mitochondria in more detail.

Mitochondrial FL Uptake. To determine the time-dependence of FL uptake, mitochondria were incubated with 100 μM FL for different time periods (Fig. 2). Mitochondrial uptake of FL was rapid, with an initial rate of 60 pmol/mg protein·min, and reached equilibrium after 5 min. Uptake decreased at increasing medium osmolarity, indicating that FL levels represent true uptake into the mitochondrial matrix, and little binding (< 10%) was observed (data not shown). The intramitochondrial volume was 2.5 ± 0.02 μl/mg of protein (n = 3) as determined by the method described by Halestrap (1989). At equilibrium, the uptake of 100 μM FL was, on average, 57 pmol/mg of protein, which suggests an intramitochondrial concentration of 23 μM. This indicates that FL does not accumulate in mitochondria; however, it should be emphasized that the organic anion needs to be transported against a steep electrochemical gradient of −180 to −220 mV (LaNoue and Schoolwerth, 1979). After 45 s, FL uptake tends to deviate from initial linear uptake; therefore, an early time point of 15 s was chosen to study concentration-dependent uptake. Figure 3 presents the uptake of different concentrations of FL, measured with and without coadministration of 12.5 mM phenylsuccinate, a specific inhibitor of the α-ketoglutarate carrier. The uptake of FL was concentration-dependent and sensitive to phenylsuccinate. At high concentrations, the nonlinear uptake paralleled the phenylsuccinate-inhibited uptake, indicating saturation of the transport system. Fitting of the equally weighted phenylsucc-
cinate-corrected uptake data to the Michaelis-Menten equation resulted in an apparent $K_m$ of $1.3 \pm 0.3$ mM with a $V_{max}$ of $260 \pm 26$ pmol/mg protein·15 s ($R^2 = 0.989$).

**Effect of Inhibitors on Mitochondrial FL Uptake.** In addition to the $\alpha$-ketoglutarate carrier, other metabolite anion transporters of the mitochondrial inner membrane may be involved in FL uptake. To investigate whether additional carriers are involved, mitochondria were coincubated for 15 s with 100 $\mu$M FL and 10 mM of a substrate or specific inhibitor (Fig. 4). The affinities of substrates for the mitochondrial transporters are in the low millimolar range; therefore, a concentration of 10 mM was chosen to ensure significant inhibition. Several of the anions that were tested are intermediates of the Krebs cycle. Metabolism of these substrates may lead to rapid changes in matrix volume and, consequently, may alter FL uptake. Therefore, the experiments were performed in the presence of the metabolic blocker antimycin A (2 $\mu$M). This concentration completely inhibits oxygen consumption and substrate metabolism in renal cortical mitochondria (Chen and Lash, 1998). Antimycin A did not affect the uptake of FL in the presence of phenylsuccinate, suggesting that phenylsuccinate is not subjected to mitochondrial metabolism. As mentioned above, phenylsuccinate significantly inhibited the uptake of FL, indicating the involvement of the $\alpha$-ketoglutarate transporter. Concentration-dependent inhibition of 100 $\mu$M FL uptake by phenylsuccinate over the concentration range of $10^{-7}$ to $10^{-2}$ M revealed a $-\log IC_{50}$ value of 4.0 $\pm$ 0.4 (Table 1). Butyramalonate, a specific inhibitor of the dicarboxylate carrier, had no effect on the uptake of FL. However, coincubation with citrate or phosphoenolpyruvate, which are both substrates for the tricarboxylate carrier, significantly inhibited FL uptake to the same extent of approximately 35%. The log concentration-inhibition analysis of the inhibition of FL uptake by citrate (range $10^{-7}$ to $10^{-2}$ M) revealed a $-\log IC_{50}$ value of 4.6 $\pm$ 0.4 (Table 1). Furthermore, malate and succinate, substrates for the dicarboxylate, tricarboxylate, and $\alpha$-ketoglutarate transporters, affected the uptake of FL significantly. A substrate for the monocarboxylate carrier, pyruvate had no effect on FL uptake. Glutamate and aspartate, however, produced a significant inhibition. Both metabolic substrates are transported by the glutamate-aspartate exchanger, whereas glutamate is a substrate for the glutamate carrier as well. Glutamate exhibited a 20-fold higher inhibitory potency against FL uptake as compared with aspartate (Table 1), whereas maximum inhibition by both substrates was in the same order of magnitude. In addition, a combination of phenylsuccinate, glutamate, and citrate produced the strongest inhibition, resulting in a total reduction of FL uptake of 66%.

**Effect of Anionic Drugs on Mitochondrial FL Uptake.** The effect of other anionic drugs on the mitochondrial uptake of FL was tested to determine whether the interaction with mitochondrial transporters is specific for FL or may also occur for other organic anions. Mitochondria were incubated for 15 s with 100 $\mu$M FL in the presence of several different drugs. Concentrations were chosen based on previously reported findings for these drugs on mitochondrial functioning (Tokumitsu et al., 1977; Rumbach et al., 1989; Tune et al., 1989; Vessey et al., 1996). Valproate, indomethacin, and salicylate produced a significant inhibition of FL uptake of 27, 19, and 23%, respectively (Fig. 5). Cephaloglycin and cephaloridine, however, had no effect on the uptake of FL. Even concentrations up to 5 mM cephaloridine produced no reduction in FL uptake.

**Discussion**

Anionic drugs tend to accumulate in PTC during active renal secretion. The results of this study with permeabilized cells suggest that the organic anion FL associates with intracellular compartments. Uptake in these cells was sensitive to an inhibitor of renal organic anion uptake, probenecid, suggesting the existence of specific binding or an active up-

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**Fig. 4.** Effect of 10 mM of a substrate or specific inhibitor on the uptake of 100 $\mu$M FL in renal cortical mitochondria. Mitochondria were incubated for 15 s at 10°C without treatments (control) and after preincubation with phenylsuccinate (PS), butyramalonate (BM), citrate (CI), phosphoenolpyruvate (PEP), malate, succinate, pyruvate, glutamate (GL), aspartate, or a combination of PS, CI, and GL. Data are mean $\pm$ S.E. of at least four different isolations. Statistical comparisons: significantly different from controls, $^* P < .05$, $^* * P < .01$.

**Fig. 5.** Effects of 100 $\mu$M valproate, 1 mM cephaloglycin, 1 mM cephaloridine, 100 $\mu$M indomethacin, and 500 $\mu$M salicylate on the uptake of 100 $\mu$M FL in renal cortical mitochondria. Mitochondria were incubated for 15 s at 10°C. Data are mean $\pm$ S. E. of at least three different isolations. Statistical comparisons: significantly different from controls, $** P < .01$.
take mechanism in intracellular compartments. Previous investigations have shown mitochondrial compartmentation; however, the mechanism of interaction between FL and mitochondria remained to be explained (Masereeuw et al., 1994). Indirect association studies suggested that FL is taken up by one of the metabolite anion carriers of the mitochondrial inner membrane (Masereeuw et al., 1996). In this study, we performed direct uptake experiments of FL with rat renal cortex mitochondria. The uptake of FL in isolated mitochondria was time- and concentration-dependent and sensitive to phenylsuccinate, indicating that the α-ketoglutarate carrier may mediate this transport. Fitting the phenylsuccinate-corrected data to the Michaelis-Menten equation revealed an apparent $K_m$ of 1.3 ± 0.3 mM. This value is in good agreement with the inhibitory constant ($K_i$) of 1.1 ± 0.8 mM as determined for FL on succinate uptake (Masereeuw et al., 1996), and it provides evidence that the same transport system mediates succinate and FL uptake. Some of the anion carriers in the mitochondrial inner membrane have fairly broad, and sometimes overlapping, substrate specificities (Fonyo et al., 1976; LaNoue and Schoolwerth, 1979; Schoolwerth and LaNoue, 1985; Gullans and Hebert, 1996). Therefore, more than one system could be involved in the uptake of the anionic drug. To further characterize additional transport system(s) involved, FL uptake was studied in the absence and presence of various substrates and specific inhibitors. Figure 6 shows a summary of our results, and presents the mechanism by which FL may be transported into renal cortical mitochondria. Malate, succinate, citrate, phosphoenolpyruvate, glutamate, and aspartate significantly reduced the uptake of FL. Glutamate and aspartate are both transported by a glutamate-aspartate exchanger, and glutamate also is a substrate for the glutamate carrier. Because maximum inhibition values by glutamate and aspartate were in the same order of magnitude, we suggest that there is an interaction of FL with the glutamate-aspartate exchanger rather than with the glutamate carrier, although partial involvement of the latter cannot be ruled out. Pyruvate and butyraldehyde produced no interaction, whereas the combination of phenylsuccinate, citrate, and glutamate produced the strongest inhibition. Taken together, our findings indicate that the α-ketoglutarate transporter, the tricarboxylate carrier, and the glutamate-aspartate exchanger mediate concomitantly the uptake of FL.

The inhibitory potencies of phenylsuccinate, citrate, aspartate, and glutamate were all in the micromolar range. The concentration of 10 mM that was used to determine the involvement of the metabolite anion transporters produced maximum inhibition. Because several transporters mediate the uptake of FL, the addition of 10 mM of a substrate or specific inhibitor for one transport system is not able to completely block FL uptake. Determination of the kinetic characteristics of one metabolite carrier in isolated mitochondria is difficult because of the overlapping specificities of the multiple carrier systems present in this functional unit. To better understand the mechanism of interaction between FL and phenylsuccinate, aspartate, glutamate, or citrate, experiments with isolated transport systems are needed. An approach would be the purification and functional reconstitution of the transporter of interest into a liposomal system (Bolli et al., 1989; Kaplan and Mayor, 1993).

It has been shown previously that metabolic substrates, such as pyruvate, lactate, fatty acids, and intermediates of the Krebs cycle, stimulate the uptake of FL in renal cortex slices (Nikiforov and Ostretsova, 1992). However, the stimulatory effects cannot be explained by activation of ATP production only. Most metabolic substrates that stimulated the uptake in renal proximal tubules are not able to exchange directly with extracellular organic anions. Therefore, Nikiforov and Ostretsova (1992) suggested that these substrates are converted by gluconeogenesis into products that may exchange for FL. Regarding the results of our investigation, it may be speculated that the increased cellular uptake of FL was at least in part due to an enhanced mitochondrial uptake. Metabolic substrates are easily taken up by mitochondria where they may be converted to malate via the gluconeogenic pathway. Transport of malate to the cytoplasm may occur via exchange with FL mediated by either the α-ketoglutarate, tricarboxylate, and/or glutamate-aspartate carriers.

This study showed that the model substrate, FL, is actively taken up in renal cortex mitochondria. A similar interaction possibly may be expected for other anionic drugs that are known to accumulate in renal PTC and might explain their nephrotoxic potential. The xenobiotics, valproate, indomethacin, and salicylate, affected the mitochondrial uptake of FL, suggesting that these drugs interact with mitochondrial transporters. The interaction between valproate and mito-

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Fig. 6. Mechanism of mitochondrial uptake of FL. The involvement of six major mitochondrial anion transporters of the inner membrane was investigated. These transporters are 1) monocarboxylate carrier, 2) dicarboxylate carrier, 3) tricarboxylate carrier, 4) α-ketoglutarate carrier, 5) glutamate-aspartate exchanger, and 6) glutamate carrier. Functional characteristics are adapted from references (Fonyo et al., 1976; LaNoue and Schoolwerth, 1979; Schoolwerth and LaNoue, 1985; Gullans and Hebert, 1996). FL may be a substrate for the tricarboxylate carrier, the α-ketoglutarate transporter, and the glutamate-aspartate exchanger.
chondrial anion carriers is in good agreement with earlier findings (Rumbach et al., 1989). Indomethacin and salicylate are known to cause uncoupling of mitochondrial oxidation and phosphorylation (Tokumitsu et al., 1977; Vessey et al., 1996), whereas an interaction with mitochondrial transporters has not been described. However, the cephalosporins, cephaloglycin and cephaloridine, produced no inhibition of FL uptake. This is in contrast with previous studies in which an inactivation of mitochondrial transporters was shown. Exposure of isolated mitochondria to cephaloridine produced a reversible reduction in succinate uptake, whereas in vivo exposure to cephalosporins led to acylation of the transporters (Tune et al., 1989, Tune, 1993). The discrepancy between these studies and our findings with FL uptake may be explained by the involvement of other carrier systems and/or species differences.

In conclusion, the mitochondrial uptake of FL is mediated by mitochondrial metabolite anion carriers, which are normally involved in the transport of endogenous metabolic substrates. The present data reveal that the organic anion FL is a substrate for at least three metabolite carriers, viz., the α-ketoglutarate transporter, the tricarboxylic acid carrier, and the glutamate-aspartate exchanger.

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References


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