Drug–nutrient interactions: inhibition of amino acid intestinal absorption by fluoxetine

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Fluoxetine is one of the most widely used antidepressants and nowadays it is also being used to manage obesity problems. In our laboratory we demonstrated that the drug inhibited sugar absorption (Monteiro *et al.* 1993). The aim of the present work was to determine the effect of fluoxetine on intestinal leucine absorption. Using a procedure of successive absorptions *in vivo* the drug diminished amino acid absorption by 30 % ($P < 0.001$). Experiments *in vitro* in isolated jejunum also revealed a reduction in leucine uptake of 37 % ($P < 0.001$). In both cases fluoxetine only affected mediated transport without altering diffusion. In a preparation enriched in basolateral membrane, fluoxetine inhibited the Na^+,K^+-ATPase (*EC* 3.6.1.37) activity (55 %; $P < 0.001$) in a non-competitive manner with an inhibition constant (K_i) value of 0·92 mM. Leucine uptake by brush-border membrane vesicles was diminished by the drug (a reduction of 48 % was observed at 30 s, $P < 0.001$); only the apical Na^+-dependent transport system of the amino acid was modified and the inhibition was non-competitive. Leucine uptake in the presence of lysine indicated that transporter B was involved. These results suggest that fluoxetine reduces leucine absorption by its action on the basolateral and apical membrane of the enterocyte; the nutritional status of the patients under drug treatment may be affected as neutral amino acid absorption is decreased.

Fluoxetine: Drug–nutrient interactions: Intestinal absorption

Fluoxetine (FLX), a selective serotonin reuptake inhibitor, is one of the most widely used antidepressants (Bauman, 1996). Moreover, FLX has been reported to promote weight reduction (O'Kane *et al.* 1994), to treat obsessive-compulsive disorders (Fulton & McTavish, 1995) and the premenstrual syndrome (Steiner *et al.* 1995). Its leptogenic action could be attributed to an effect on the central nervous system (Angel *et al.* 1988). Compared with other tri- and tetracyclic antidepressants, FLX has more gastro-intestinal side-effects such as nausea, soft stools and diarrhoea. When administered for prolonged periods, adverse effects such as intestinal interactions with gastro-intestinal components might occur. In fact, a previous study of this antidepressant found that FLX diminished intestinal sugar absorption (Monteiro *et al.* 1993) and therefore the study of its effect on another nutrient group, such as amino acids, could be of interest.

Amino acid transport is more complex than sugar absorption as many transporters are involved in this process. At least six different transport systems for neutral amino acids have been identified on the brush-border membrane of enterocytes (Ganapathy *et al.* 1994); two Na^+-independent and the rest Na^+-dependent systems.

We chose to use a neutral, essential amino acid, L-leucine. The aim of the present study was to investigate the effect of FLX on leucine absorption in the small intestine using *in vivo* and *in vitro* methods. This included the study of the uptake of L-leucine using isolated jejunum, the measurement of fluxes across the gut wall, basolateral Na^+,K^+-ATPase (*EC* 3.6.1.37) activity and uptake by brush-border membrane vesicles (BBMV).

Materials and methods

Chemicals

FLX (Lilly Indiana Madrid, Spain) was kindly donated. L-[1-^{14}C]leucine (specific radioactivity 1850–2220 MBq/mmol) was purchased from Amersham International plc (Amersham, Bucks., UK). L-Leucine, mannitol, ouabain,

Abbreviations: BBMV, brush-border membrane vesicles; FLX, fluoxetine; V, initial uptake rate; V/S, initial uptake rate/substrate concentration.

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imidazole, Triton X-100, ATP, bovine serum albumin and Percoll were bought from Sigma Chemical Co. (St Louis, MO, USA). Ammonium molybdate and 1-amino-2-naphthol-4-sulfonic acid were acquired from Fluka (Buchs, Switzerland). Scintillators Formula 989 (for liquid samples) and Optiphase 'Hisafe' 3 (for solid samples) were from Du Pont de Nemours (Brussels, Belgium) and LKB (Milton Keynes, Beds., UK) respectively. Cellulose nitrate filters (type HAWP 0·45 μm, 25 mm diameter) were from Millipore (Madrid, Spain). All other chemicals were commercial products of reagent grade. The FLX concentration used was 0·3 mM (this concentration is in the range used in animals), except in kinetic studies. Radioactivity was determined on a liquid scintillation counter (Wallac 1409; Pharmacia, Barcelona, Spain).

Animals

Animals were handled according to the European Council Legislation 86/609/EEC on experimental animal protection. Male Wistar rats (180–200 g body weight) were obtained from the Center of Applied Pharmacology (CIFA, Pamplona, Spain). The intestinal segment used was the jejunum and the animals were anaesthetized with subcutaneous doses of sodium pentothal (60 mg/kg).

Amino acid absorption in vivo

The procedure of successive absorptions in a closed circuit has already been described elsewhere (Ponz *et al.* 1979). Briefly, a jejunal loop of 150 mm, starting 50 mm distal to the ligament of Treitz, was isolated between two glass cannulas, while the mesenteric vasculature of the loop was maintained. At each end of the loop an incision was made, and a cannula was introduced into it. The other sides of the cannulas were connected to tygon tubes and the tubes were also connected to a perfusion system, equipped with a constant-flow electric pump (Microperpex, model 2123, LKB Produkter, Milton Keynes, Beds., UK). The loop was reinserted in the abdominal cavity and the abdomen was closed. The anaesthetized animal and all the perfusion solutions were kept at 37°. The loop was first rinsed with 50 ml saline solution (140 mM-NaCl, 10 mM-$KHCO_3$, 0·4 mM-KH_2PO_4, 2·4 mM-K_2HPO_4, 1·2 mM-$CaCl_2$ and 1·2 mM-$MgCl_2$; pH 7·4) and then perfused with the same buffer containing 0·5 mM-L-leucine and 0·37 kBq/ml [^{14}C]leucine for six successive 10 min intervals. The effect of the drug was measured by including it at a concentration of 0·3 mmol/l in the second of these perfusion periods. Perfusions with or without FLX were performed in twelve animals. The volume perfused was 10 ml. Between perfusion periods the intestine was washed with saline for 5 min. Leucine absorption was estimated as the difference between the amino acid in the solution before and after perfusion. Results are expressed as μmol leucine/cm per 10 min. To evaluate both transport components, mediated and non-mediated transport, 60 mM-L-alanine (Lerner & Larimore, 1986) was added, with 0·5 mM-leucine in the perfusate. The NaCl concentration in the buffer was reduced to maintain the osmolality. Twelve animals were perfused, with or without FLX.

Amino acid uptake in isolated jejunum

About 200 mm of the jejunum was quickly excised and rinsed with an ice-cold saline solution. To perform uptake experiments, pieces of the intestine, each weighing about 50 mg, were incubated and continuously oxygenated (O_2–CO_2, 95 : 5, v/v) in the saline solution described earlier. The incubations took place at 37° and 0–4°, with 0·5 mM-L-leucine and 0·37 kBq/ml radioactive substrate with or without FLX, for 5, 15, 30 and 45 min. At the end of the incubation the tissues were washed in an ice-cold saline solution, blotted carefully to remove excess moisture, weighed wet and extracted by shaking for 24 h in 100 mM-HNO_3 (0–4°). Fifteen animals were used for each incubation temperature, 37° and 0–4°. The number of intestinal pieces taken from each animal ranged from sixteen to twenty-four. Two or three pieces were incubated in 10 ml saline solution for each experimental condition and the mean of those results was taken as one uptake measurement. Values are expressed as μmol L-leucine/ml intracellular water. Extracellular space was measured by incubating the tissue for 20 min with saline solution containing 0·74 kBq/ml [^{3}H]PEG-4000 (Amersham International). Following extraction the tissues were dried at 110° for 48 h, then re-weighed. Tissue water was calculated as the difference between wet and dry weights. FLX had no effect either on extracellular space, or on intracellular water (results not shown).

Measurement of L-*leucine flux across the gut*

The intestine was opened along the mesenteric border and mounted as a flat sheet in an Ussing-type chamber (Naftalin & Curran, 1976). The bathing solutions of the mucosal and serosal tissue surfaces contained 0·5 mM-L-leucine and were maintained at 37°. The tissue was preincubated for 20 min. Mucosal-to-serosal fluxes were measured by adding 1·48 kBq/ml of radioactive L-leucine to the mucosal side. Every 20 min, samples of 0·2 ml were taken from the serosal side and radioactivity determined. Results are the means of twelve animals.

Measurement of basolateral Na^+,K^+-*ATPase activity*

A fraction enriched in basolateral membrane was obtained using the method of Del Castillo & Robinson (1982). A jejunal segment was removed and opened longitudinally. The mucosa was scraped off and homogenized in 250 mM-sucrose and 2 mM-Tris–HEPES (pH 7·2). The suspension was centrifuged at 1500 ***g*** for 10 min, the precipitate was resuspended in 2 M-sucrose and centrifuged once more at 12 500 ***g*** for 10 min. The supernatant fraction, diluted in seven volumes of distilled water, was centrifuged at 35 000 ***g*** for 15 min and the precipitate was mixed with a solution of Percoll (116·7 ml/l), 2 M-sucrose and 2 mM-Tris (pH 7·2) and centrifuged at 50 800 ***g*** for 1 h. The Percoll fraction was centrifuged at 50 800 ***g*** for another 1 h and the

final pellet was resuspended in the required volume of 2 M-sucrose and 2 mM-Tris–HEPES (pH 7·2) to obtain a protein concentration of 4–6 mg/ml. The preparation obtained had a Na^+,K^+-ATPase activity 15 (SEM 0·5) times greater than that of the original tissue. Nevertheless, slight contamination with brush-border membrane was observed. The activity of sucrose α-glucosidase (*EC* 3.2.1.48), a marker enzyme for the brush-border membrane (Van Beers *et al.* 1995), was reduced 16-fold compared with that of the original tissue. The ATPase activity was determined by the method of Jorgensen (1975) modified by Hardcastle *et al.* (1986). Ouabain (3 mmol/l) was used to inhibit Na^+,K^+-ATPase and its activity was calculated as the difference in ATPase activity in the absence and presence of ouabain. Twelve basolateral membrane preparations were used, each taken from five animals.

For the determination of the Na^+,K^+-ATPase kinetic parameters in the presence of FLX, Na^+,K^+-ATPase was assayed with different ATP concentrations (0·1–10·0 mmol/l). The concentration of the drug in the incubation medium ranged between 0·0 and 1·0 mmol/l. Data were analysed by the Lineweaver–Burk plot.

Preparation of brush-border membrane vesicles

The brush-border membrane was prepared by the method of Shirazi-Beechey *et al.* (1990). Briefly, this involved suspending the everted intestine in a buffer containing 2 mM-HEPES/Tris (pH 7·1) and 100 mM-mannitol, and stirring in a Vibro-Mixer (model E-1; Sorvall, Newtown, CT, USA) for 3 min. A $MgCl_2$ solution was added to achieve a final concentration of 10 mmol/l and the preparation was stirred for 30 min at 0–4°. After centrifuging several times, the final pellet of the vesicle preparation was suspended in the desired volume of 300 mM-mannitol, 0·1 mM-$MgSO_4$ and 10 mM-Tris–HEPES buffer (pH 7·4) (load solution), using a no. 27 gauge needle. The final protein concentration range was 7–10 mg/ml. The enrichment of the specific activity of sucrose α-glucosidase was 10·5 (SEM 1·0)-fold. The contamination with the basolateral membrane was very low (Na^+,K^+-ATPase activity was reduced 20-fold with respect to homogenate). Protein was determined according to the method of Bradford (1976), with bovine albumin as standard. For BBMV experiments fifteen preparations were made, each one from five animals.

Transport studies

Time-course studies of amino acid uptake were performed using a rapid filtration technique (Hopfer *et al.* 1975). A portion of the BBMV preparation (5 µl) was added to the incubation medium (45 µl) to start the experiments. The total L-leucine uptake was divided into three components, namely Na^+-dependent, Na^+-independent and diffusion. In order to separate these systems, three parallel time-course experiments were necessary: (1) under a Na^+ gradient (0 mM in : 100 mM out) and at 25°, to measure the total uptake; (2) in the absence of Na^+ and with a K^+ gradient (0 mM in : 100 mM out) and at 25°, to measure Na^+-independent uptake plus diffusion; (3) in the presence of a Na^+ gradient (0 mM in : 100 mM out) and at 0–4°, to evaluate the diffusion component.

Na^+-dependent uptake, with or without FLX, was calculated as the difference in uptake between the first two conditions. The difference between the second and third conditions was assumed to be equivalent to Na^+-independent uptake. The final concentration of the incubation medium was: 10 mM-Tris–HEPES buffer (pH 7·4), 0·1 mM-$MgSO_4$, 100 mM-mannitol, 100 mM-NaSCN or 100 mM-KSCN, 0·5 mM-L-leucine and 50 µM-radiolabelled substrate. At the stated times the incubation was stopped by the addition of 3 ml ice-cold solution containing 150 mM-KSCN and 10 mM-Tris–HEPES buffer (pH 7·4). The suspension was poured onto a prewetted Millipore filter that was washed twice with 3 ml ice-cold stop solution, dissolved in scintillator 'Hisafe 3' and counted. Non-specific binding to the filter was subtracted from the uptake value.

Kinetics

The initial leucine uptake rate as a function of external leucine concentration can be used to calculate the kinetic parameters. A time of 3 s was chosen to measure initial uptake. Some leucine experiments were determined in the presence of an inward Na^+ gradient and others in a K^+ gradient. These conditions should enable separation of Na^+-dependent-carrier-mediated transport from Na^+-independent plus the diffusion pathway.

Data were represented by an Eadie–Hofstee plot. In this plot, used when studying kinetics of transporters, the initial uptake rate (V) *v.* the initial uptake rate/substrate concentration (V/S) is represented (Harig *et al.* 1989). Analysis of the data to estimate the kinetic parameters was performed by a non-linear fitting of the initial uptake rate to the Michaelis–Menten equation using the program Sigma Plot (version 3.02; Jandel Scientific, Jandel Corporation, Palo Alto, CA, USA).

Competitive studies

L-Leucine uptake by BBMV was measured in the presence and absence of 10 mM-lysine. The experiments were performed at 25° and in a Na^+ gradient (0 mM in : 100 mM out) condition. The uptake was measured at 30 s. L-Leucine uptake measured in the presence of an excess of lysine is mainly through system B (Munck, 1995).

Statistics

Results are expressed as means with their standard errors. Data were analysed statistically using Student's *t* test. Differences were considered significant if the *P* value was < 0.05.

Results

Effect in vivo *of fluoxetine*

The effect *in vivo* was measured over six successive 10 min perfusion periods in the same animal, perfusing a saline

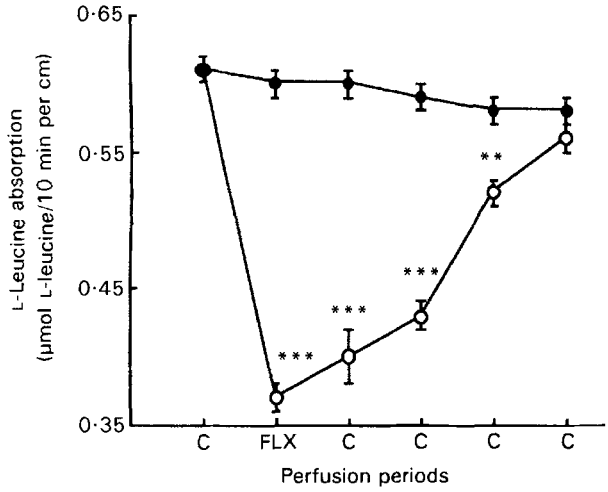

Fig. 1. Effect of the antidepressant fluoxetine (FLX) on absorption of L-leucine by the intestine *in vivo*. Six perfusion periods of 10 min were carried out in the same animal. During the control period (C; ●) 0·5 mM-L-leucine was perfused. During the FLX periods (○) perfusion was with either 0·5 mM-L-leucine (periods 1, 3, 4, 5 and 6) or 0·5 mM-L-leucine + 0·3 mM-FLX (period 2). For details of procedures, see pp. 440–441. Values are means for twelve animals, with their standard errors represented by vertical bars. Mean values were significantly different from control, $^{**}P < 0.01$, $^{***}P < 0.001$.

solution with 0·5 mM-L-leucine during each period except in the second, where the amino acid and the drug were perfused simultaneously. FLX reduced amino acid absorption (Fig. 1) during the second period of perfusion ($P < 0.001$). This inhibition was diminished in subsequent periods when only leucine was perfused and returned to the control value in period six. To find out which of the two pathways, diffusion or mediated-transport, was affected by FLX, the effect of the drug was measured in the presence of a high concentration (60 mM) of another amino acid (L-alanine) that shares the same transporters with L-leucine. In the presence of alanine, leucine carrier-mediated transport was practically abolished, and any absorption was mediated by diffusion. The mediated transport was calculated as the difference between absorption without L-alanine and absorption with L-alanine. The antidepressant only affected the carrier-mediated transport system (Fig. 2).

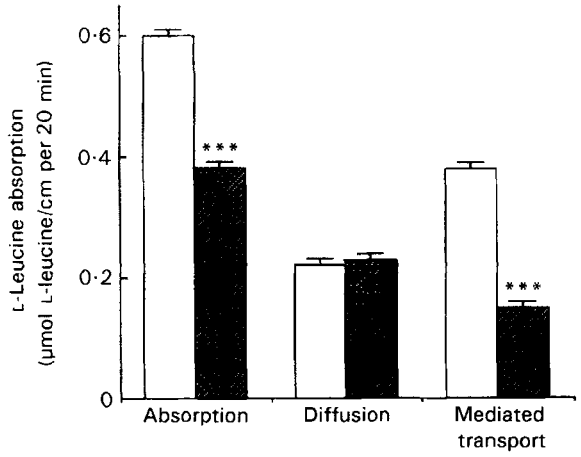

Fig. 2. Effect of fluoxetine (FLX) on the mediated and non-mediated transport of L-leucine *in vivo*. Animals were perfused with 0·5 mM-L-leucine with (▨) or without (□) 0·3 mM-FLX. To measure diffusion, 60 mM-L-alanine was added to the perfusate. Mediated transport was taken as the difference between absorption and diffusion. For details of procedures, see pp. 440–441. Values are means for twelve animals, with their standard errors represented by vertical bars. Mean values were significantly different from control, $^{***}P < 0.001$.

Amino acid uptake and mucosal to serosal flux

FLX inhibited leucine uptake significantly by 37–42% (Table 1). The effect was observed at all incubation times (5–45 min) and was not affected by the time of the incubation. On the other hand, leucine uptake was not altered by the drug at 0–4°, when only diffusion was measured. These findings, in agreement with those obtained *in vivo*, indicated that only mediated transport was reduced by FLX. Mucosal-to-serosal fluxes were also inhibited by FLX; under control conditions, L-leucine mucosal-to-serosal flux was 0·31 (SEM 0·03) μmol/cm^2 per h, with FLX this flux diminished to a value of 0·19 (SEM 0·02) μmol/cm^2 per h ($P < 0.001$).

Effect of fluoxetine on Na^+,K^+-ATPase activity

The effect of FLX on the Na^+, K^+-ATPase activity was measured in a preparation of purified basolateral membrane with little brush-border membrane contamination. Na^+,K^+-ATPase activity was measured indirectly using ouabain as an inhibitor. FLX reduced the activity of this enzyme by 55% (Fig. 3) but did not affect that of the ouabain-insensitive ATPase (non-Na^+,K^+-dependent ATPase). Kinetic studies were performed to clarify the inhibitory effect of the antidepressant. As shown in Fig. 4, the Lineweaver–Burk plot analysis demonstrated that FLX inhibited the enzyme activity in a non-competitive manner, indicating that FLX does not compete with ATP for its active site. For control, an apparent maximum velocity (V_{max}) of 654·0 μmol P_i/mg protein per h and an apparent Michaelis constant (K_m) of 2·1 mmol/l were recorded. The apparent inhibitor constant (K_i) value, determined by the replot of slopes of the Lineweaver–Burk Plot, was 0·92 mmol/l.

Time courses of L-leucine uptake by brush-border membrane vesicles

As shown in Fig. 5, there was an overshoot of L-leucine uptake in the presence of a Na^+-gradient (100 mM in: 0 mM out) with a peak at about 30 s. At this time the Na^+-dependent pathway contributed 91% of the total uptake. The uptake recorded in a K^+-gradient was higher than the level of the passive diffusion of L-leucine. This indicated the presence of a Na^+-independent pathway for L-leucine. This transport system measured at 30 s represented 5% of the total uptake. Diffusion, measured at the same experimental time, accounted for the remaining 4%. FLX only affected the Na^+-dependent transport system, without altering the other components.

In kinetic experiments the initial uptake rate of leucine through the Na^+-dependent transport system was measured

Table 1. Uptake of L-leucine by isolated jejunal rings in the presence or absence of fluoxetine (FLX) at 37° and 0–4°† (Mean values with their standard errors for samples from fifteen animals)

	L-Leucine uptake (μmol L-leucine/ml intracellular water)							
	37°				0–4°			
	Control		FLX		Control		FLX	
Time (min)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
5	1·23	0·04	0·77***	0·03	0·29	0·01	0·27	0·01
15	1·79	0·07	1·10***	0·04	0·36	0·02	0·39	0·01
30	2·30	0·08	1·35***	0·07	0·50	0·03	0·51	0·01
45	3·12	0·10	1·59***	0·09	0·56	0·03	0·56	0·02

Mean values were significantly different from those for the control, ***$P < 0.001$.
† For details of procedures, see pp. 440–441.

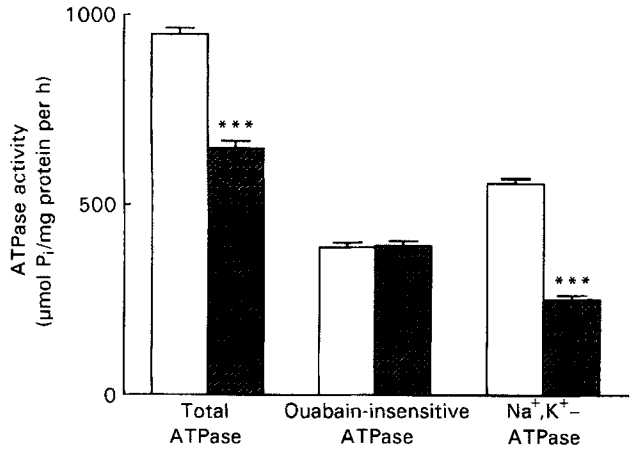

Fig. 3. Effect of the antidepressant fluoxetine (FLX) on the activity of basolateral ATPase. A preparation enriched in basolateral membrane was incubated at 37° for 30 min with (▨) or without (□) 0·3 mM-FLX. Ouabain (3 mmol/l) was used to inhibit Na^+,K^+-ATPase and its activity was calculated as the difference between total ATPase and ATPase measured in the presence of ouabain, expressed as μmol P_i/mg protein per h. For details of procedures, see pp. 440–441. Values are means for twelve preparations, with their standard errors represented by vertical bars. Mean values were significantly different from control, ***$P < 0.001$.

at seven different leucine concentrations, ranging from 0·1 to 5·0 mmol/l and four FLX concentrations, 0·3, 0·5, 0·7 and 1·0 mmol/l. The slope of the lines obtained in the presence of FLX did not differ from that of the control ($P < 0.05$), indicating a non-competitive inhibition (Fig. 6). FLX and leucine bind to different places of the Na^+-dependent transport system.

By non-linear fitting of the data (r 0·998), an affinity constant of 2·22 (SEM 0·1) mmol/l was obtained. In control conditions a V_{max} of 356·00 (SEM 9·0) pmol L-leucine/mg protein per 3 s was recorded.

Competitive studies

In addition to the B transporter, $B^{0,+}$ and $b^{0,+}$ systems are present in the brush-border membranes of enterocytes (Pickel *et al.* 1993; Mailliard *et al.* 1995). One method of inhibiting the $B^{0,+}$ system is to measure leucine uptake in the presence of an excess of lysine (Campa & Kilberg, 1989; Munck, 1989; Magagnin *et al.* 1992).

In the presence of 10 mM-L-lysine, FLX reduced L-leucine uptake (control: 314 (SEM 12) *v.* FLX: 162 (SEM 6) pmol L-leucine/mg protein per 30 s to the same extent as when the basic amino acid was not present in the incubation medium (control: 471 (SEM 19) *v.* FLX: 212 (SEM 7) pmol L-leucine/mg protein per 30 s). This means that FLX affected the brush-border Na^+-dependent neutral amino acid transport system (B) and had no effect on $B^{0,+}$.

Discussion

FLX is one of the most widely used drugs today. The drug is a highly specific inhibitor of active serotonin reuptake into presynaptic nerve endings in the brain (Wong *et al.* 1975). In addition to its use as an antidepressant, it is also prescribed in obesity. Studies in animals have reported that FLX reduces food intake (Wong & Yen, 1985) mainly suppressing the intake of high-carbohydrate diets (Luo & Li, 1990). Clinical studies have also shown it to promote weight reduction in obese subjects (Wise, 1992) and in obese patients with type 2 diabetes (Connolly *et al.* 1995); it even minimizes weight gain after smoking cessation (Spring *et al.* 1995). FLX action in obesity has been attributed to its anorexic effect mediated by serotonin (Blundell & Hill, 1989). However, since FLX is an oral drug, a direct action on the intestine, such as interference with nutrient absorption, may also be possible. We therefore studied the effects of this antidepressant on the intestinal absorption of a neutral amino acid, L-leucine. This nutrient is transported at the apical membrane by a combination of the B, $B^{0,+}$, $b^{0,+}$ and ASC systems (Ganapathy *et al.* 1994; Brandsch *et al.* 1995; Matthews *et al.* 1996) and also by the recently described y^+L system (Jun *et al.* 1995). Studies carried out *in vivo* using the successive intestinal absorption technique, in which drug perfusion of the tissue was only carried out in the second perfusion period, showed that there was a strong inhibition of leucine absorption (30%), but this was restored to the control value with time. Because leucine absorption by diffusion was not affected by this drug (Fig. 2), the inhibition observed was due to an effect on mediated transport systems. This phenomenon was also observed in

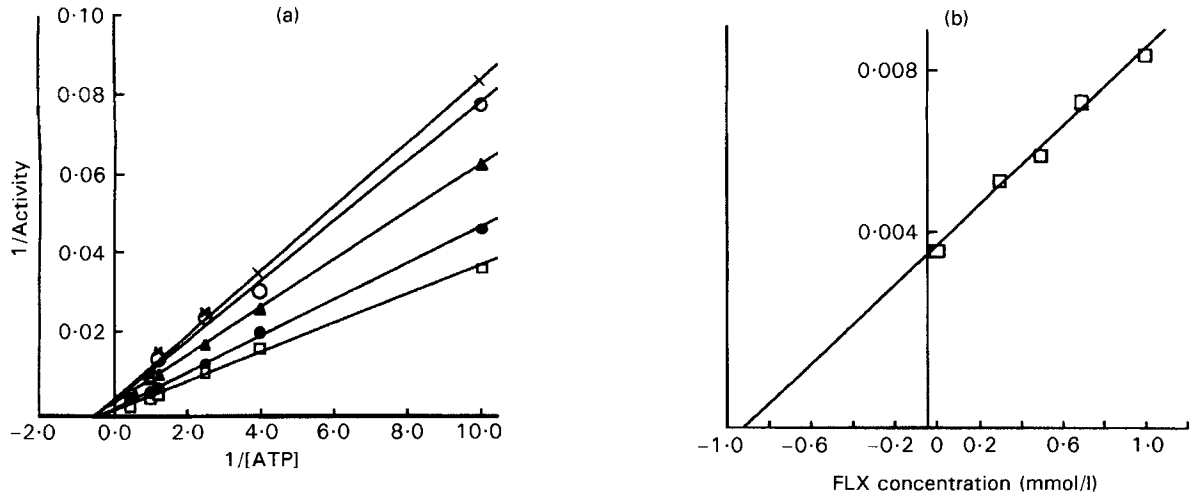

Fig. 4. Analysis of the inhibitory effect of fluoxetine (FLX) on the activity of Na^+,K^+-ATPase. (a) Analysis by the Lineweaver–Burk plot. Final concentrations of FLX were: 0 (control; □), 0·3 (●), 0·5 (▲), 0·7 (○) and 1·0 (×) mmol/l. Each value represents the mean of twelve results. (b) The re-plot of the slopes of the Lineweaver–Burk plot.

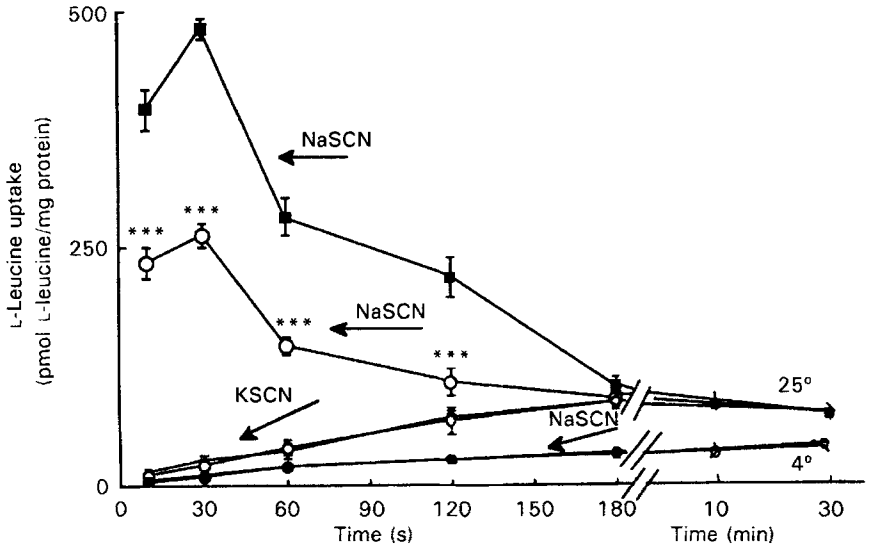

Fig. 5. Time course of L-leucine uptake by brush-border membrane vesicles in the presence (○) or absence (■) of 0·3 mM-fluoxetine. Vesicles were loaded with 300 mM-mannitol, 0·1 mM-$MgSO_4$ and 10 mM-HEPES–Tris buffer (pH 7·4). The incubation medium contained: 100 mM-NaSCN or KSCN, 100 mM-mannitol, 0·1 mM-$MgSO_4$, 10 mM-HEPES–Tris buffer (pH 7·4) and 0·5 mM-L-leucine. Uptake studies were performed at 25° and 4°. For details of procedures, see pp. 440–441. Values are means with their standard errors indicated by vertical bars for fifteen results made in triplicate. Control and fluoxetine results for KSCN and at 4° are superimposed. Mean values were significantly different from control, $^{***}P < 0.001$.

the study of the effect of cefroxadine and cephaloglycine on the absorption of amino acids (Barcina *et al.* 1988; Mendizabal *et al.* 1990, 1991).

In order to confirm the inhibition, we carried out experiments *in vitro* to determine the effects of the drug on the transport of leucine using two preparations, intestinal rings and transepithelial flow. The results using intestinal rings showed a strong and rapid inhibition of amino acid uptake by FLX (37%, $P < 0.001$). This was observed within 5 min and did not increase with time (Table 1). This inhibition appears to indicate the effect of FLX on mediated transport because it disappeared when the uptake was measured at 0–4°, a temperature at which mediated transport systems are inoperative. (McCloud *et al.* 1996). Results of transepithelial flow of the amino acid showed that mucosal-to-serosal flux was decreased. FLX may act directly on leucine uptake, or have an indirect effect. This latter possibility could be through FLX inhibition of serotonin reuptake, since serotonin significantly diminishes the uptake and the steady-state tissue accumulation of leucine (Salvador *et al.* 1996) and may also function as a neurotransmitter and a local paracrine modulator in the gastrointestinal tract (Gershon *et al.* 1985).

The data reveal that mediated transport of leucine is inhibited by FLX and therefore research on its effect on the Na^+,K^+-ATPase seemed logical. This enzyme, anchored on the basolateral membrane, plays a definitive role in the control of transmembrane Na and K gradients in the cell and thus is of importance in the Na^+-dependent transport

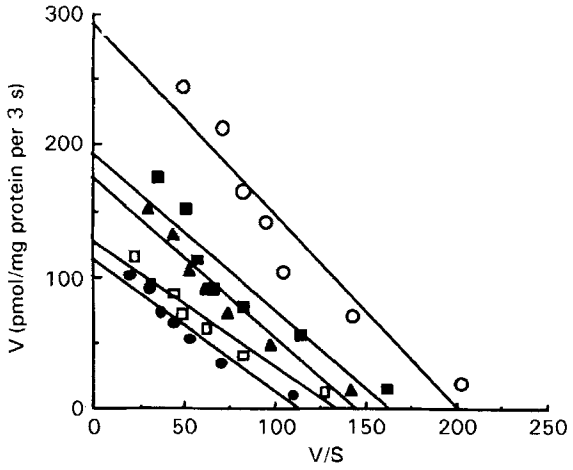

Fig. 6. Kinetic analysis of the inhibitory effect of fluoxetine (FLX) on Na^+-dependent L-leucine uptake. The figure shows an Eadie–Hoffstee plot of the Na^+-dependent uptake measured at 3 s and 25° (○), Control; (■), 0·3 mM-FLX; (▲), 0·5 mM-FLX; (□), 0·7 mM-FLX; (●), 1·0 mM-FLX; substrate concentration ranged from 0·1 to 5·0 mM. The plot is of initial uptake rate (V) *v.* initial uptake rate/substrate concentration (V/S). Values are means for fifteen results.

system of leucine. Fig. 3 shows that FLX decreased the activity of this enzyme without affecting any other ATPase. This is a non-competitive enzyme inhibition and affects only the Na^+-dependent amino acid transport system (Dixon & Hokin, 1980). The K_i value obtained for FLX is low compared with that of some antibiotics that also inhibit Na^+,K^+-ATPase activity. This would be related to the extent to which the absorption is inhibited. For cefaclor we found a K_i of 4·0 mmol/l, and a 1·0 mM antibiotic concentration produced a 15% inhibition of the absorption (Idoate *et al.* 1996). A concentration of 0·3 mM-FLX reduced leucine absorption by 30% (Fig. 1).

To find out if FLX acted on the transport system of the apical membrane, BBMV were prepared. In this preparation FLX only affected the Na^+-dependent transport system (Fig. 5), as determined by subtracting the uptake in the presence of a K^+ gradient from that in the presence of a Na^+ gradient, both measured at 25°. Kinetic studies confirmed that this inhibition was non-competitive (Fig. 6), since the affinity constant of the amino acid for the transporter was not affected by the drug. Conversely, the maximum velocity of transport was reduced as the concentration of FLX was increased. These results indicated that FLX had a direct effect on apical Na^+-dependent L-leucine transport. When the uptake of 0·5 mM-L-leucine in the presence of 10 mM-lysine was measured, the inhibition caused by the drug was similar in the presence and absence of lysine. This indicates that $B^{0,+}$ transporter is not affected and system B is involved.

Our results show that FLX has an action on the intestinal absorption of nutrients. We can conclude that FLX affects leucine transport by interacting with basolateral Na^+,K^+-ATPase and the apical Na^+-dependent transport system (system B). The Na^+,K^+-ATPase inhibition implies that transport of nutrients coupled with Na is reduced. Glucose is transported coupled with Na and a decrease in its absorption would be beneficial in obesity. On the other hand, a reduction in amino acid absorption may affect the nutritional status of patients under FLX therapy.

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