Amino Acids (2000) 19: 667–685

Amino
Acids

Detailed study of the different taurine uptake systems of colon LoVo MDR and non-MDR cell lines

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Accepted August 16, 1999

Summary. In human, physiological taurine requirement is partly dependent on nutrition. Study of the human carcinoma LoVo cells shows the presence of a high and a low affinity taurine uptake. Besides them, a diffusion system has been found. A detailed analysis of the properties of the three systems is presented. A comparison of LoVo chemosensitive cells, and LoVo chemoresistant (MDR) cells which overexpress the multidrug transporter P-glycoprotein, shows that the only difference between the two cell types belong to the kinetic properties of the high and low affinity taurine uptake systems.

Keywords: Amino acids – Taurine uptake – MDR cells – Imino acid carrier – LoVo cell lines

Introduction

Taurine (2-aminoethanesulfonic acid) is a naturally occurring β-amino acid that is ubiquitously and abundantly distributed in many tissues (Hayes and Sturman, 1981; Huxtable, 1992). In human intestinal mucosa, taurine ranks as the most or one of the most abundant free amino acid, representing 38% in duodenum and 18% in colon of the free amino acid pool (Ahlman et al., 1993; O'Flaherty et al., 1997). Taurine has been shown to play an important role in many physiological events, including osmoregulation, antioxidation, membrane stabilisation, neuromodulation, cardiac protection, retinal and brain development among others (Chesney, 1985; Wright et al., 1986; Sturman, 1987; Huxtable, 1989). Taurine synthesis in human can not completely fulfil the physiological requirement. Humans are therefore partly dependent of nutritional sources (Chesney, 1985).

In mammalian, taurine is taken up by intestine. Extensive research using brush-border membrane vesicles or brush-border membrane of intestinal epithelium has shown the presence of transport systems for the uptake of dietary taurine in mammals (Miyamoto et al., 1989; Munck and Munck, 1992;

Sharafuddin and Nassar 1993; Munck et al., 1994; Barada et al., 1997; O'Flaherty et al., 1997). By contrast, taurine transport in human intestine has only been characterised using cultured non-MDR cell lines derived from human colon carcinomas: HT 29 (Tiruppathi et al., 1992) and Caco 2 (Satsu et al., 1997). All these studies have detailed the properties of the high affinity uptake. Few data have been reported concerning diffusion. Pratically nothing is known about the properties of the low affinity uptake, when such a system has been reported.

Cancer treatments reveal the existence of two types of tumours: those, which are sensitive to usual chemotherapeutic agents and those, which are resistant to these agents. One of the most studied resistant phenotype is linked to the overexpression in the plasma membrane of resistant cells of P-glycoprotein (P-gp), a 170-kDa transporter. P-gp has been shown to actively extrude numerous chemically and functionally unrelated chemotherapeutic agents out of cells (Multidrug resistance – MDR phenotype) (Gottesman and Pastan, 1988, 1993; Gottesman et al., 1992; Leveille-Webster and Arias, 1995). We present here a detailed analysis of the different taurine uptake systems found in the human colon carcinoma LoVo cells. We have compared LoVo MDR and non-MDR cell lines to test what could be the effects of the plasma membrane changes on a transport localised in these membranes.

Materials and methods

Chemicals

[^{3}H] taurine (29 Ci/mmol) was from DuPont-NEN (NET 541). Unlabeled taurine and L-arginine hydrochloride were from Janssen Chimica. Taurine analogues were from Sigma excepted guanidinoethylsulfonate (Toronto Research Chemicals, North York-Canada), β-alanine (Merck), and N-acetyl taurine previously synthesised as reported (Petegnief et al., 1995). Doxorubicin and verapamil were from Sigma. All other chemicals were of analytical grade.

Cell culture

The parental non-MDR LoVo cell line and its multidrug resistant counterpart (LoVo/DX) were obtained from Pharmacia & Upjohn (Milan, Italy). From the parental drug sensitive cell line (LoVo S), two variants were selected, which differed morphologically: the "LoVo S fusoid" and the "LoVo S small cells" (Lelong-Rebel et al., unpublished data). Preliminary results show that these two cell lines differ by the degree of differentiation. The original LoVo/DX cells (MDR) were derived from the parental drug sensitive LoVo cells as described by Grandi et al. (1986) and were shown to overexpress P-glycoprotein (Grandi et al., 1986). The LoVo/DX cells that we have received have kept the same resistance levels towards different chemotherapeutic drugs, as previously found by Grandi et al. Moreover, these cells became sensitive to these drugs in presence of verapamil, a P-glycoprotein revertant. The LoVo/DX cells were originally resistant to 0.1 μg/ml doxorubicin. These cells were further selected in resistance up to 0.4 μg/ml doxorubicin and named LoVo Dox.

The three LoVo cell lines were grown as monolayer cultures in 75 cm^2 vented flasks (Costar n° 3376) at 37°C in a 5% CO_2 atmosphere in Dulbecco modified Eagle medium/Ham F12 nutrient mixture 1/1 (DMEM/HamF12, Seromed n° F-4815) supplemented with 50 U/ml penicillin and 50 μg/ml streptomycin, 5% (v/v) heat inactivated foetal calf serum

(FCS – Dutscher, Bischwiller-France), 365.30 mg/l L-glutamine and 55 mg/l sodium pyruvate. Osmolarity of the final medium was 305 mOsm. LoVo Dox culture medium contained also 0.4 μg/ml doxorubicin. Mycoplasmas were routinely checked with Hoechst A 33258.

For the uptake experiments, cells were seeded in 35 mm Petri dishes (Costar n° 3035) and grown in 2 ml of their respective culture medium up to the required density. Culture media were changed every other day. Last medium change was made 24 to 48 hours before the taurine uptake measurement.

Proliferation of LoVo cells in presence of increasing concentrations of FCS

Approximately 200 cells were seeded in 35 mm Petri dishes in 2 ml of their respective culture medium. 12 hours later, the culture medium was removed and replaced by 2 ml of DMEM/Ham F12 supplemented with 0.5%, 5%, 10%, or 20% (v/v) FCS (+0.4 μg/ml doxorubicin for LoVo Dox cells). Addition of various amounts of serum did not affect medium osmolarity. Culture media were changed every 2 days. Proliferation was determined by Crystal Violet staining as described by Kueng et al. (1989), stain being desorbed with acetic acid 33% in double distilled water. Cell viability was determined by MTT test, formazan being solubilized with ethanol (Lelong and Rebel, 1998a).

Taurine uptake measurement

Taurine uptake was measured the method of Lelong and Rebel (1998b). Briefly, culture media were removed and replaced by 3 ml of CO_2 independent medium (CIM) (GIBCO, n° 18045-054). Cells were preincubated for 5 or 10 min in a rotator water bath at 37°C. Then, 100 μl of CIM containing a mixture of 0.4 μCi [^{3}H] taurine and either 1.33 μM (high affinity), 200 μM (low affinity) or 2 mM (non saturable uptake) non radioactive taurine were added to the preincubation medium. After 10 min incubation, the medium was removed, cells were washed three times with ice cold NaCl 0.9% and frozen at −20°C. Cells were homogenised in 1 ml of NaOH 0.1 N. Aliquots were taken for protein determination according to Lowry et al. (1951) and for radioactivity measurements by scintillation counting with Aquasol (Packard).

To study Na^+ and Cl^- dependence of taurine uptake, cells were preincubated for 10 min and incubated for 10 min in air in Krebs-Ringer buffer (KR) or in a modification of KR without Na^+ and Cl^-.

Taurine efflux measurements

Confluent cultures grown on Petri dishes were loaded in their respective culture media with 0.4 μCi [^{3}H] taurine for 2 h. Cells were then treated as follows: a first set of dishes was washed three times with ice cold NaCl 0.9% then frozen (control); other sets of dishes were washed three times with 3 ml of CIM, incubated for 10 min in 3 ml of CIM containing either 1.33 μM, 200 μM or 2 mM unlabeled taurine, then washed three times with ice cold NaCl 0.9% and frozen. Cells were homogenised in 1 ml of NaOH 0.1 N. Aliquots were removed for radioactivity and protein determination. The efflux data were calculated as the differences (in %) between the radioactivity per mg of protein in the control cells versus the incubated cells.

DNA determination and osmotic pressure measurement

For DNA determination, 1 ml of double distilled water was added to the frozen dishes instead of NaOH 0.1 N. Then, cells were sequentially thawed and frozen (Rago et al., 1990). DNA was measured using a modification of the Rago buffer as described by Lelong and Rebel (1998b).

Osmotic pressure was measured with a Roebling osmometer. The osmolarity of all the media and physiological buffers used were in the physiological range (290–310 mOsm).

Noticeable was the fact that increasing taurine concentrations in CIM did not affect medium osmolarity (osmolarity of CIM, CIM + 200 μM taurine and CIM + 2 mM taurine was 300, 302 and 304 mOsm respectively).

Statistical analysis

Each experimental measurement was performed at least in quadruplicate. The kinetic parameters of taurine uptake were calculated by linear regressions of the Eadie-Hoffstee plots and confirmed by a non-linear regression program on Kaleidagraph (version 3.0.8 D, Abelbeck Software). Kinetic parameters are means ± SEM of three such determinations. Results are expressed as means ± SEM of taurine uptake (pmol/min/mg of protein). Statistical significance of the experimental results was obtained by Variance Analysis with a Fisher's test on Statview program. $P < 0.05$ was accepted as denoting statistical significance.

Results

Proliferation of LoVo cell lines as a function of FCS concentration

LoVo S fusoid proliferation was similar in DMEM/Ham F12 supplemented with 0.5, 5, 10 or 20% FCS. Proliferation of LoVo S small cells increased as a function of FCS concentration but was similar in DMEM/Ham F12 supplemented with 10 or 20% FCS. LoVo Dox proliferation increased in a dose dependent manner, being significantly higher for each FCS concentration tested. Under similar growth conditions, proliferation of LoVo S small cells was higher than that of LoVo S fusoid cells. Proliferation of the two LoVo S lines was higher than that of the LoVo Dox (Fig. 1). In addition, the type of confluence differed between the three LoVo lines. While the fusoid cells rapidly reached stable confluence at a relatively low density, the small cells reached a more instable confluence at a higher density, some cells leaving the cell layer for the medium. LoVo Dox never gave a confluent layer, more cells leaving the cell layer when density increased. Similar results were obtained when DNA or protein concentrations were used to express cell proliferation (results not shown). The protein/DNA ratio was identical for the three LoVo cell lines (0.181 ± 0.002 for the LoVo S fusoid cells; 0.180 ± 0.001 for the LoVo S small cells and 0.179 ± 0.003 for the LoVo Dox cells). This ratio remained constant in all the culture conditions.

Taurine uptake in LoVo S and LoVo Dox cells

As the protein/DNA ratio was identical for the three LoVo cell lines and independent of cell density, proteins were used to express uptake results.

Taurine uptake increased with time, being linear for at least 30 min (Fig. 2). It was greater for the LoVo Dox cells than for the 2 LoVo chemosensitive cells. Uptake was slightly higher in LoVo S small cells than in LoVo S fusoid cells. A 10 min incubation was chosen for taurine uptake determinations.

Kinetic analysis of taurine uptake showed the presence of two saturable uptake systems in the three cell lines (Fig. 3). Kinetic parameters of the two systems are indicated in Table 1. They were similar in the two LoVo S lines.

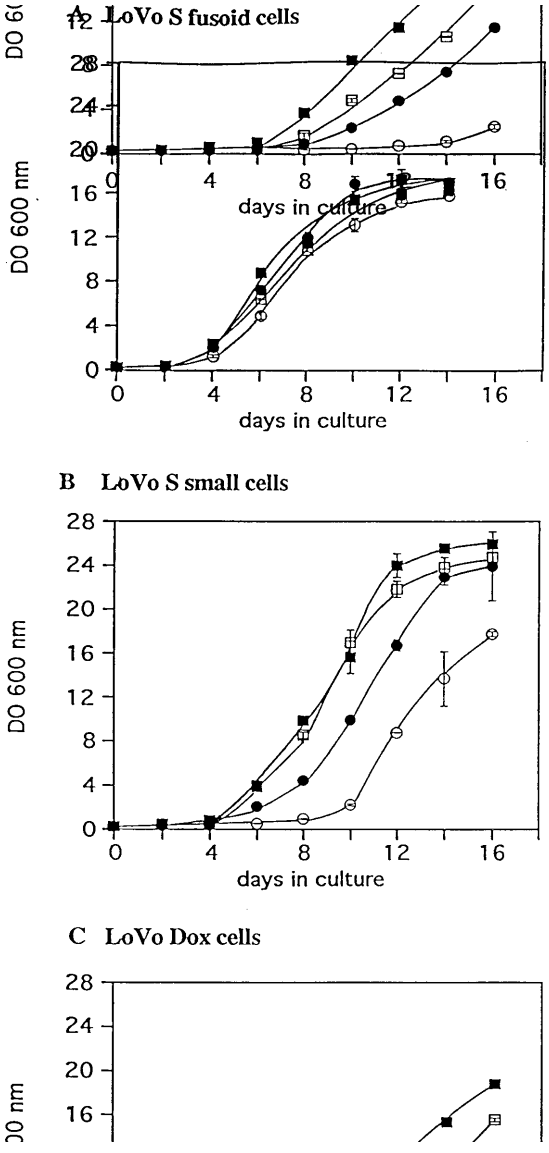

Fig. 1. Proliferation of LoVo cell lines as a function of FCS concentration. Approximately 100 cells per ml were seeded in 35 mm Petri dishes in 2 ml of DMEM/Ham F12 + 5% FCS (+0.4 μg/ml doxorubicin for LoVo Dox cells). 12 hours later, culture medium was removed and replaced by 2 ml of DMEM/Ham F12 supplemented with either 0.5% (○), 5% (●), 10% (□) or 20% (■) (v/v) FCS (+0.4 μg/ml doxorubicin for LoVo Dox cells). Culture media were changed every two days. Proliferation was determined by crystal violet staining

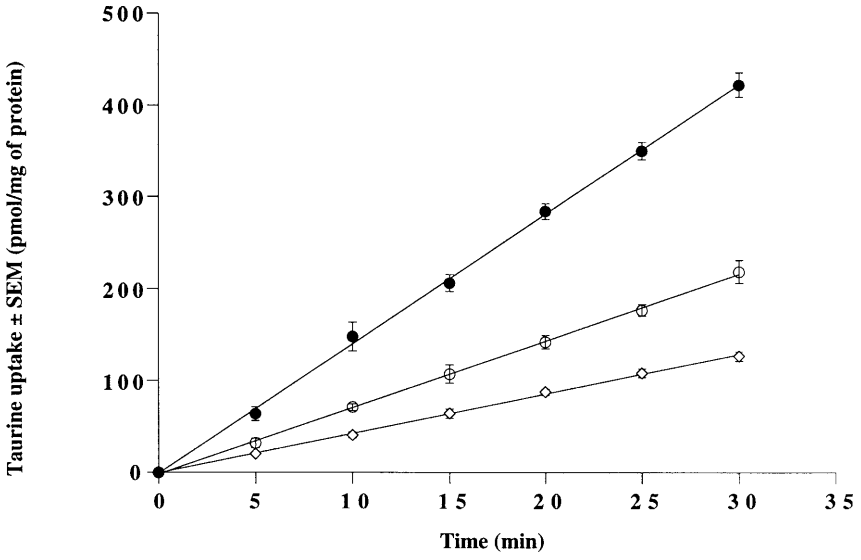

Fig. 2. Time course of taurine uptake. Confluent cells were preincubated for 10 min in CIM and then incubated for times ranging from 0.5 to 30 minutes in the presence of radiolabeled and unlabeled taurine (respectively 0.4 μCi and 1.33 μM final concentration) to measure taurine uptake as described in "Materials and methods". When not shown, error bars are smaller than symbols. ○: LoVo S small cells; ◇: LoVo S fusoid cells; ●: LoVo Dox cells. Significant differences ($p < 0.05$) between LoVo S small cells and LoVo S fusoid cells were found after 10 min incubation. Significant differences between MDR and non-MDR cells were found ($p < 0.05$ after 5 min incubation; $p < 0.01$ for longer incubations)

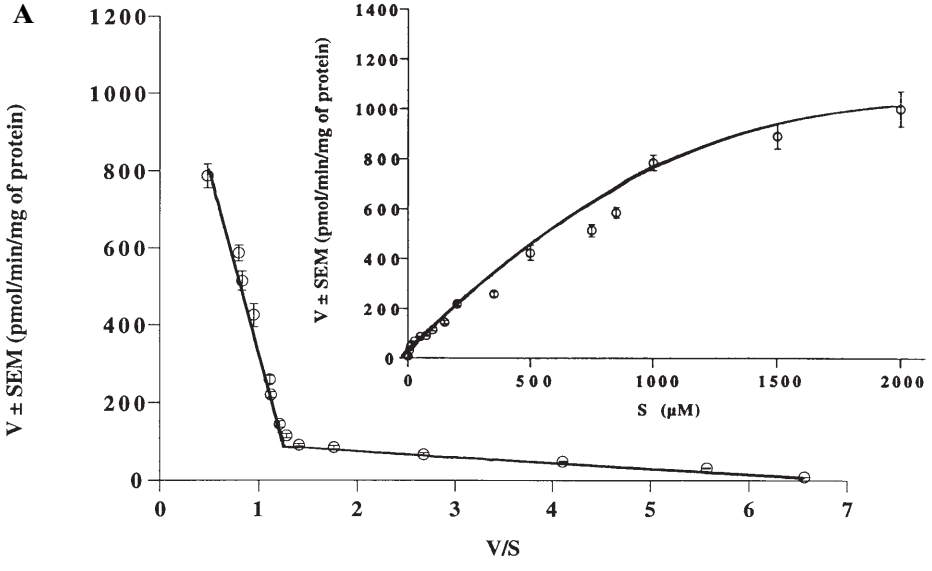

Fig. 3A

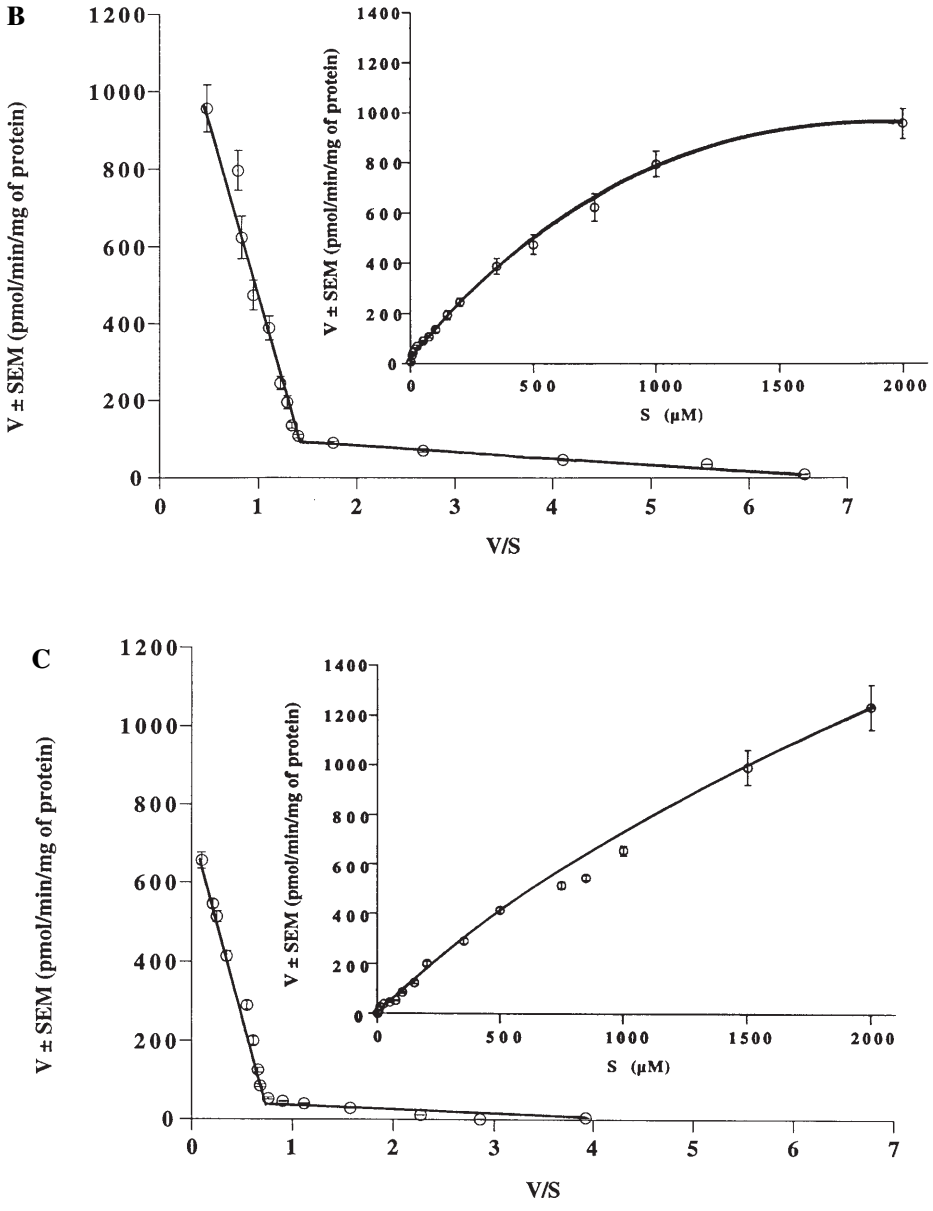

Fig. 3. Kinetic analysis of LoVo taurine uptake. Cells were grown to confluence. Initial uptake rates were determined with 10 min preincubation and 10 min incubation in CIM containing increasing concentrations of unlabeled taurine (0 up to 2 mM). Final concentration of radiolabeled taurine was maintained constant at 0.4 μCi (5 nM) for each concentration of unlabeled taurine. **A** LoVo S small cells. **B** LoVo S fusoid cells. **C** LoVo Dox cells. Results are given as Eadie-Hoffstee plots. Inset: taurine uptake as a function of taurine concentration. *V* taurine uptake ± SEM (pmol/min/mg of protein); *S* taurine concentration (μM)

Table 1. Kinetic parameters of taurine uptake

Cell lines	Vmax 1 (pmol/min/mg protein)	Km 1 (μM)	Vmax 2 (pmol/min/mg protein)	Km 2 (μM)
LoVo S small cells	110.1 ± 5.8	16.0 ± 1.1	1,212.1 ± 27.2	910.7 ± 24.1
LoVo S fusoid cells	120.8 ± 5.9	15.9 ± 1.1	1,458.8 ± 25.4	993.2 ± 25.3
LoVo Dox cells	60.8 ± 4.2 (*)	25.2 ± 1.4 (*)	704.0 ± 18.2 (*)	1,664.3 ± 25.3 (*)

Kinetic parameters were calculated by linear regression of the Eadie-Hoffstee plots and confirmed by a nonlinear regression programm on kaleidagraph (version 3.0.8, Abelbeck Software). Mean ± SEM of the kinetic parameters found in three independent determinations are shown. (*) = significant differences in the kinetic parameters between the two LoVo S and the LoVo Dox cells ($p < 0.05$).

Table 2. Basal taurine efflux present in the conditions of the uptake measurements

Cell lines	Taurine concentration in the medium (μM)	[^{3}H] taurine (cpm/μg of proteins) in cells		[^{3}H] taurine efflux (% of control)
		No incubation in CIM (control)	After 10 min incubation in CIM	
LoVo S small cells	1.33	43.73 ± 0.927	43.69 ± 0.547	0.10%
	200	43.73 ± 0.927	40.23 ± 0.756	8.00%
	2,000	43.73 ± 0.927	39.56 ± 0.570	9.53%
LoVo S fusoid cells	1.33	30.57 ± 0.754	30.54 ± 0.419	0.10%
	200	30.57 ± 0.754	30.51 ± 0.615	0.20%
	2,000	30.57 ± 0.754	29.38 ± 0.580	3.90%
LoVo Dox cells	1.33	60.25 ± 0.789	49.28 ± 0.7303	18.20% (*)
	200	60.25 ± 0.789	48.80 ± 0.759	19.00% (*)
	2,000	60.25 ± 0.789	46.08 ± 0.6571	23.51% (*)

Basal taurine efflux was measured as described in "Materials and methods". Taurine efflux was calculated as difference between radioactivity found in control cells – cells incubated 10 min, in percent of activity in control cells (100%). (*) = significant differences in efflux between cells incubated in CIM and non-incubated (control) cells ($p < 0.05$).

However, the Vmax of the two systems were reduced and the Km slightly but significantly increased in LoVo Dox cells compared to the two LoVo S lines. The presence of a non-saturable uptake was also observed when the three cell lines were incubated in the presence of high extracellular taurine concentrations (1 to 2 mM). Growing the cells in a medium supplemented with 1 mM taurine for 2 months did not affect significantly the kinetic parameters of the different uptake systems in the two LoVo sensitive cell lines and in the LoVo Dox cells. Table 2 controls represent the taurine uptake during a

2 hour incubation (see "Materials and methods"). These results confirm that the uptake was slightly greater in LoVo S small cells compared to LoVo S fusoid cells. If we take in account the high efflux, taurine uptake in LoVo Dox cells was twice greater than the one found in non-MDR cells.

In the following experiments, uptake measurements were made in the presence of 1.33 μM, 200 μM and 2 mM of unlabeled taurine to characterise respectively the high affinity, the low affinity and the non saturable uptake systems.

Table 2 shows taurine basal efflux determined under the experimental conditions used for taurine uptake. The basal efflux was much greater in the MDR cells than in their non-MDR counterparts, representing about 1/5 of the taurine taken up by the LoVo Dox cells.

Table 3. Na^+ and Cl^- dependence of taurine uptake

Uptake system	Incubation Medium	Cell lines		
		LoVo S small cells	LoVo S fusoid cells	LoVo Dox cells
High affinity	KR	12.80 ± 1.02	11.56 ± 1.11	14.18 ± 1.59
	KR-Na^+	0.47 ± 0.03 (3.7%) (**)	0.50 ± 0.06 (4.3%) (**)	0.81 ± 0.06 (5.7%) (**)
	KR-Cl^-	0.36 ± 0.03 (2.8%) (**)	0.39 ± 0.04 (3.4%) (**)	0.44 ± 0.04 (3.1%) (**)
Low affinity	KR	207.30 ± 7.36	128.11 ± 7.84	450.00 ± 41.71
	KR-Na^+	103.03 ± 5.62 (49.7%) (**)	70.59 ± 4.79 (55.1%) (**)	73.44 ± 6.22 (49.4%) (**)
	KR-Cl^-	110.28 ± 4.78 (53.2%) (**)	76.10 ± 5.48 (59.4%) (**)	81.04 ± 7.31 (53.7%) (**)
Non-saturable	KR	690.03 ± 35.09	637.83 ± 40.49	880.22 ± 36.69
	KR-Na^+	672.77 ± 38.13 (97.5%)	614.23 ± 39.43 (96.3%)	890.02 ± 47.95 (101.1%)
	KR-Cl^-	632.75 ± 36.22 (91.7%)	554.91 ± 44.83 (8.7%)	816.84 ± 41.85 (92.8%)

Cells were grown to confluence. Uptake of radiolabeled taurine (0.4 μCi final concentration) was measured with 10 min preincubation and 10 min incubation in Krebs-Ringer buffer (KR), in Krebs-Ringer without Na^+ (KR-Na^+) and in Krebs-Ringer without Cl^- (KR-Cl^-). KR contained 118.27 mM NaCl, 9.7 mM KCl, 1.95 mM $CaCl_2$, 15 mM NaH_2PO_4 (H_2O), 0.60 mM $MgSO_4$ (7 H_2O) and 5 mM D-glucose. In KR-Na^+, NaCl and NaH_2PO_4 were replaced mol per mol with LiCl and KH_2PO_4 respectively. In KR-Cl^-, NaCl, KCl and $CaCl_2$ were replaced mol per mol respectively with their respective gluconate salts. pH of the buffers was adjusted to 7.4 with KOH. The results are expressed as means ± SEM of taurine uptake (pmol/min/mg of protein). Values in brackets show taurine uptake in % of control for each uptake system. The osmolarity of all solutions used was in the physiological range (290–310 mOsm). (**) = statistical significant reductions of each uptake system compared to the respective controls (KR) for each cell line ($p < 0.01$).

Characteristics of the taurine uptake

High affinity uptake was reduced by 94–97% by incubating cells in Krebs-Ringer without Na^+ or Cl^- (Table 3). Under the same conditions, low affinity uptake was reduced by 40–51%. In contrast, non-saturable uptake was not significantly reduced under these conditions.

High and low affinity uptakes were reduced when the incubation medium osmolarity increased, showing a similar osmosensitivity. Non-saturable uptake was never affected by hyperosmolarity (Table 4).

Figure 4 shows the effects of some taurine structural analogues on the taurine uptake. Taurine, β-alanine, GES, β-guanidinopropionate and N-acetyltaurine strongly inhibited the high affinity uptake. GABA less inhibited high affinity uptake (Fig. 4A). β-alanine, GES and β-guanidinopropionate significantly reduced low affinity uptake. GABA was only a weak inhibitor. Surprisingly, the low affinity uptake was not significantly reduced by N-acetyltaurine (Fig. 4B). The two saturable uptake systems were not affected by sarcosine and α-amino acids such as α-alanine (Fig. 4A and B). None of the analogues tested affected the non-saturable uptake (Fig. 4C).

Effect of cell proliferation and density on taurine uptake

The effect of cell density on the three taurine uptake systems is complex (Table 5). Cell density affected the three uptake systems differently in the three LoVo cell lines.

LoVo Dox cells did not actively proliferate when cultured in medium supplemented with 0.5% FCS. In presence of 5% FCS, notable growth was observed after 8 days (Fig. 1). Comparison of taurine uptake in LoVo Dox grown in presence of 0.5 or 5% FCS showed no difference between the two cultures (in pmol/min/mg of proteins: 8.25 ± 0.22 and 8.11 ± 0.26 for high affinity, 254.23 ± 13.54 and 248.81 ± 15.26 for low affinity, $1{,}001.8 \pm 48.1$ and $1{,}040.3 \pm 55.11$ for non-saturable uptake in cultures grown with 0.5 or 5% FCS respectively).

Effect of P-gp substrate and P-gp inhibitor on taurine uptake

None of the three uptake systems was significantly affected by addition to the culture medium for 24 hours of doxorubicin or verapamil, alone or in combination (Table 6). Cell viability, determined using a MTT test, was unaltered by these supplementations.

Discussion

Intestinal taurine uptake has been largely studied in animal brush-border membranes (reviewed in O'Flaherty et al., 1997). It appears that the influx systems mediating intestinal taurine uptake are quite different from one

Table 4. Effect of hyperosmolarity on taurine uptake

Uptake system	Osmotic pressure (mOsm)	LoVo S small cells	LoVo S fusoid cells	LoVo Dox cells
High affinity	300	16.23 ± 0.14 (100%)	13.56 ± 0.12 (100%)	20.34 ± 0.16 (100%)
	350	13.73 ± 0.15 (84.6%)*	11.01 ± 0.10 (81.2%)*	17.07 ± 0.16 (83.9%)*
	400	10.60 ± 0.19 (65.3%)**	8.43 ± 0.11 (62.2%)**	14.22 ± 0.18 (69.9%)**
	450	8.33 ± 0.23 (51.3%)**	7.17 ± 0.12 (52.9%)**	11.76 ± 0.15 (57.8%)**
	500	5.81 ± 0.20 (35.8%)**	4.46 ± 0.08 (32.9%)**	7.42 ± 0.11 (36.5%)**
	600	2.87 ± 0.07 (17.7%)**	2.63 ± 0.09 (19.4%)**	3.72 ± 0.10 (18.3%)**
Low affinity	300	243.42 ± 13.71 (100%)	170.31 ± 10.22 (100%)	350.43 ± 15.15 (100%)
	350	207.39 ± 10.41 (85.2%)*	137.46 ± 10.10 (80.7%)*	294.36 ± 13.42 (84.0%)*
	400	157.74 ± 10.21 (64.8%)**	114.96 ± 11.24 (67.5%)**	231.98 ± 14.58 (66.2%)**
	450	127.55 ± 9.43 (52.4%)**	85.67 ± 15.42 (50.3%)**	180.12 ± 12.41 (51.4%)**
	500	88.12 ± 6.52 (36.2%)**	56.20 ± 8.61 (33.0%)**	126.15 ± 10.36 (36.0%)**
	600	53.80 ± 5.69 (22.1%)**	41.56 ± 7.56 (24.4%)**	77.80 ± 6.43 (22.2%)**
Non-saturable	300	812.32 ± 27.28 (100%)	595.28 ± 24.39 (100%)	958.54 ± 34.94 (100%)
	350	808.26 ± 28.45 (99.5%)	593.49 ± 20.13 (99.7%)	943.20 ± 29.85 (99.5%)
	400	804.20 ± 24.12 (99.0%)	591.71 ± 21.92 (99.4%)	934.58 ± 34.32 (99.4%)
	450	803.38 ± 23.54 (98.9%)	589.92 ± 22.74 (99.1%)	921.16 ± 26.54 (99.1%)
	500	799.32 ± 25.87 (98.4%)	585.76 ± 20.20 (98.4%)	913.49 ± 22.18 (98.7%)
	600	795.26 ± 24.86 (97.9%)	580.99 ± 23.81 (97.6%)	903.90 ± 31.25 (98.3%)

Confluent cells were preincubated for 5 min in CIM adjusted to the desired osmolarities with a solution of sorbitol 1 M (prepared in CIM) and 1.33 μM (high affinity uptake) or 200 μM (low affinity uptake) or 2 mM (non saturable uptake) unlabeled taurine. Then, 100 μl of CIM containing 0.4 μCi [^{3}H] taurine (5 nM) were added to the preincubation medium and the cells were incubated for 10 min. The results are expressed as means ± SEM of taurine uptake (pmol/min/mg of protein). Values into brackets show taurine uptake in % of control. Significant differences compared to the controls (300 mOsm): * = $p < 0.05$; ** = $p < 0.01$.

animal species to another. To understand the mechanisms mediating intestinal taurine uptake in man, some studies have been performed on human intestinal tumour cell lines.

Two saturable taurine uptake mechanisms were found in the three cell lines studied. With regard to the Km and Vmax values observed (Huxtable, 1989; 1992; Ganapathy and Leibach, 1994; O'Flaherty et al., 1997), it appears that the first one was a high affinity and low capacity uptake mechanism, the second being a low affinity and a high capacity uptake mechanism. The presence of a low affinity uptake system was never reported in intestinal brush-border membranes or in Caco-2 or HT29 cell lines. This certainly because this system can be clearly observed only in the presence of 200 μM of external taurine, a concentration much higher than that used in previous studies (50–100 μM). The two saturable uptake systems appeared to be highly dependent on Na^+ and Cl^- as the other intestinal taurine transport systems characterised so far in animal intestine (reviewed in O'Flaherty et al., 1997) or intestinal tumour cell lines (Tiruppathi et al., 1992; Satsu et al., 1997). However, the Na^+ and Cl^- dependence of the low affinity system seemed to be less strict than that of the high affinity system. Sensitivity of the high and low

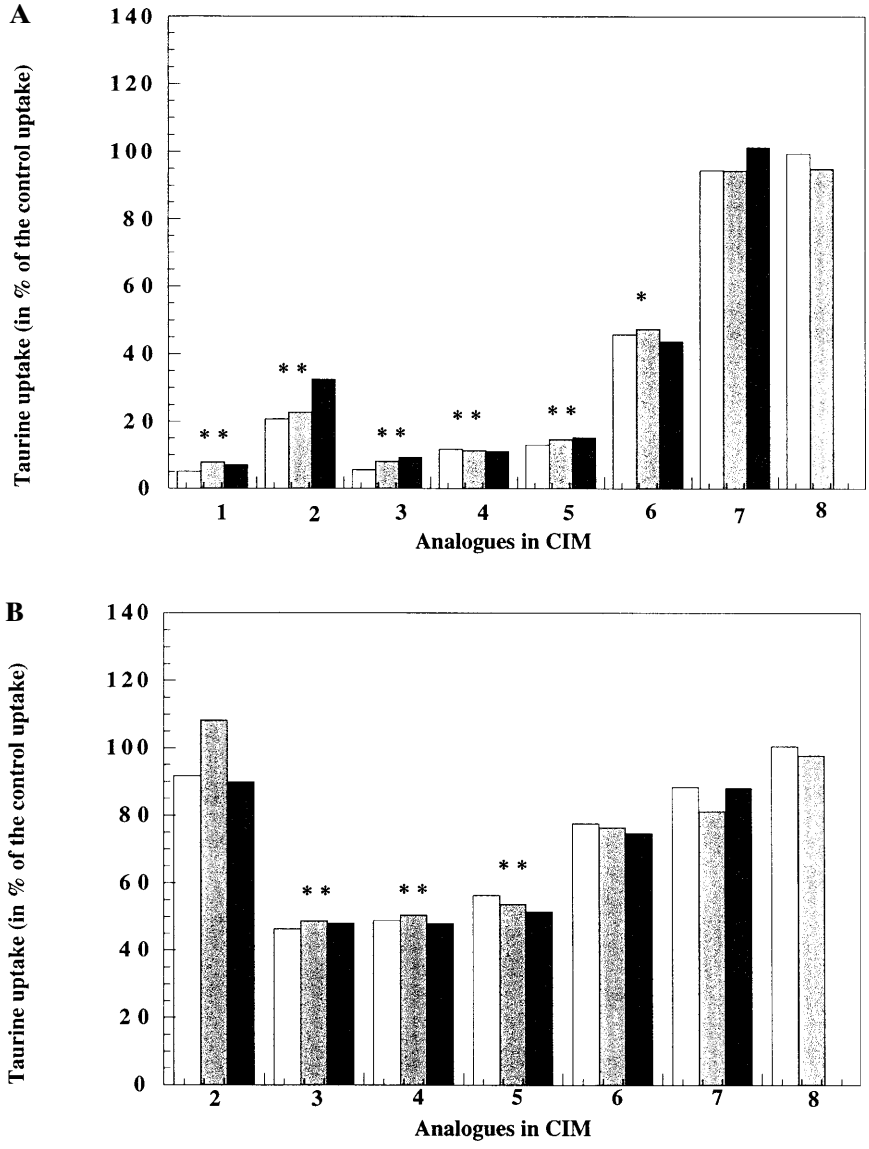

Fig. 4. Effects of taurine analogues on the high affinity (**A**), the low affinity (**B**) and the non-saturable (**C**) uptake systems. Cells were grown to confluence. Uptake of taurine was measured with 5 min preincubation and 10 min incubation in the presence or absence of 1 mM of the different unlabeled compounds in CIM (**A**) or CIM + 200 μM unlabeled taurine (**B**) or CIM + 2 mM unlabeled taurine (**C**). The analogues used were: 1. taurine; 2. N-acetyltaurine; 3. β-alanine; 4. GES; 5. β-guanidinopropionate; 6. GABA; 7. α-alanine; 8. sarcosine. Uptake levels of the controls ± SEM (pmol/min/mg of protein): **A**. 12.48 ± 0.75; 11.09 ± 0.81; 13.49 ± 0.97 for LoVo S small cells, LoVo S fusoid and LoVo Dox cells respectively; **B**. 195.14 ± 12.66; 182.18 ± 13.78; 213.39 ± 16.04 for LoVo S small cells, LoVo S fusoid and LoVo Dox cells respectively; **C**. 791.30 ± 24.42; 565.37 ± 31.21; 913.37 ± 22.88 for LoVo S small cells, LoVo S fusoid and LoVo Dox cells respectively. The effect of 1 mM unlabeled taurine was not tested on the low affinity and non-saturable uptakes as certainly at that external taurine concentration low affinity uptake and diffusion coexist. □: LoVo S small cells; ▒: LoVo S fusoid cells; ■: LoVo Dox cells. SEM were always smaller than 10%. Statistical significant reductions of the uptake in the three cell lines compared to the respective controls: ** = $p < 0.01$; * = $p < 0.05$

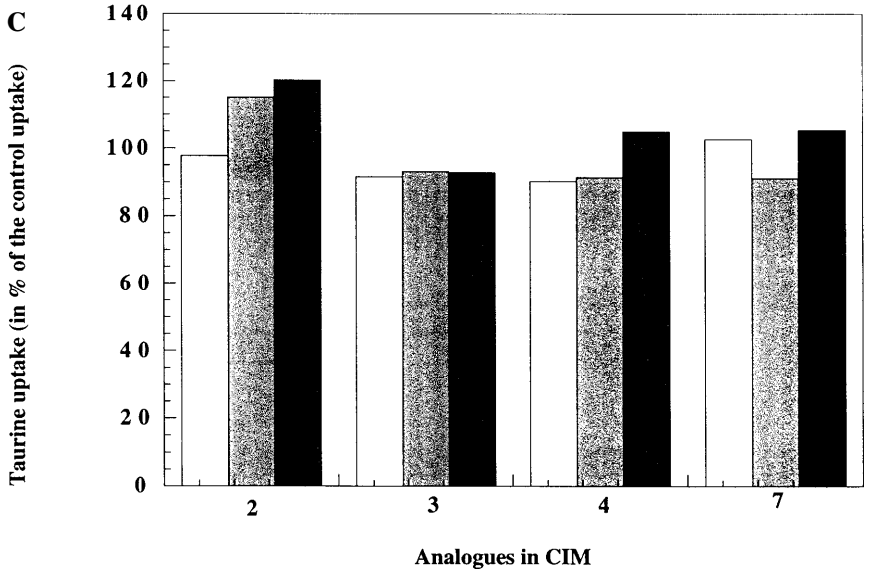

Fig. 4C

affinity uptakes to hyperosmolarity confirms that they are transport systems. Various β-amino acids reduced high and low affinity taurine transport, suggesting that they were selective for β-amino acids. The inhibition percentages of the β amino acids tested for the high affinity uptake were in the same range as has been generally found with the same concentrations of these compounds in other cell types (Huxtable, 1989; 1992; Ganapathy and Leibach, 1994; Petegnief et al., 1995). The same analogues were less inhibitory on the low affinity system. In particular, N-acetyltaurine, a very efficient inhibitor of the high affinity uptake (Petegnief et al., 1995), had no activity on the low affinity uptake system. This shows that N-acetyltaurine uptake could perhaps be used to discriminate between the high affinity, the low affinity and the diffusion taurine uptake systems. This could be useful to get an idea of the overlapping of the different uptake systems when increasing the taurine concentration in the medium.

In adult rat small intestine (Munck et al., 1994) and chicken small intestine (Lerner and Karcher, 1978), some taurine uptake is mediated by the imino acid carrier. The imino transporter is a chloride independent system inhibited by sarcosine or proline (Munck et al., 1994). The strong chloride dependence of LoVo taurine uptake as well as the complete absence of inhibition by sarcosine shows that human cells did not take up taurine via an imino acid transporter. This confirms the results of Satsu et al. (1997) who observed that 5 mM proline did not affect taurine uptake by Caco-2 cells. Sharafundin et Nassar (1993) postulated the existence in rat small intestine of a Na^+-independent carrier-dependant uptake system. The nearly complete inhibition of LoVo cell high affinity system in the absence of Na^+ ions, excluded the possible existence of such a system.

Table 5. Effect of cell density on the taurine uptake systems

	High affinity uptake		Low affinity uptake		Non-saturable uptake	
	High density	Low density	High density	Low density	High density	Low density
LoVo S small cells	5.07 ± 0.16 (*)	9.61 ± 0.13 (*)	89.36 ± 4.83	93.43 ± 5.16	390.49 ± 28.71 (*)	741.52 ± 21.92
LoVo S fusoid cells	3.06 ± 0.14 (*)	6.68 ± 0.30	66.44 ± 4.04 (*)	136.05 ± 5.84	360.24 ± 20.08 (*)	465.31 ± 21.56
LoVo Dox cells	7.10 ± 0.24	6.20 ± 0.30	132.88 ± 6.55 (*)	238.58 ± 6.42	420.28 ± 25.03 (*)	1,275.85 ± 28.10

Cells were grown either to confluence or to low density as described in "Materials and methods". Uptake of taurine was measured with 5 min preincubation and 10 min incubation in CIM. The results are expressed as means ± SEM of taurine uptake (pmol/min/mg of protein). (*) = significant reductions in uptake levels at high cell density compared to those measured in low density cultures ($p < 0.05$).

Table 6. Effect of P-gp substrate and P-gp inhibitor on taurine uptake

Uptake system	P-gp substrate	Taurine uptake (pmoles/min/mg of protein) in:		
		LoVo S small cells	LoVo S fusoid cells	LoVo Dox cells
High affinity	Control	16.94 ± 0.31	13.67 ± 0.23	20.79 ± 0.35
	Verapamil	16.92 ± 0.14	13.58 ± 0.31	20.35 ± 0.33
	Doxorubicin	16.89 ± 0.20	13.51 ± 0.32	20.31 ± 0.35
	Doxorubicin + Verapamil	16.41 ± 0.25	13.34 ± 0.30	20.12 ± 0.34
Low affinity	Control	275.32 ± 15.25	150.25 ± 10.87	412.32 ± 31.12
	Verapamil	266.74 ± 11.00	148.38 ± 11.41	408.56 ± 30.28
	Doxorubicin	264.54 ± 18.41	146.56 ± 10.52	404.17 ± 30.85
	Doxorubicin + Verapamil	251.32 ± 16.54	140.86 ± 10.23	395.25 ± 28.92
Non-saturable	Control	706.54 ± 30.40	491.39 ± 26.45	923.45 ± 38.45
	Verapamil	714.28 ± 24.22	490.24 ± 27.82	921.82 ± 39.78
	Doxorubicin	705.35 ± 32.82	487.65 ± 21.23	915.88 ± 36.69
	Doxorubicin + Verapamil	711.23 ± 30.38	480.36 ± 24.72	925.51 ± 39.98

Nearly confluent cells were grown for 24 hours in DMEM/Ham F12 + 5% FCS supplemented with: 1. vehicle (0.1% DMSO), 2. 0.4 μg/ml doxorubicin, 3. 10 μM verapamil, 4. 0.4 μg/ml doxorubicin + 10 μM verapamil. After 2 washes with CIM at 37°C, taurine uptake was determined as described in "Materials and methods" except that CIM contained either: 1. vehicle (0.1% DMSO). 2. 0.4 μg/ml doxorubicin. 3. 10 μM verapamil. 4. 0.4 μg/ml doxorubicin + 10 μM verapamil. The results are expressed as means ± SEM of taurine uptake (pmol/min/mg of protein).

Beside the high and low affinity uptakes, a non-saturable uptake system is present in the three LoVo cell lines. Recently published studies suggest that some diffusion pathway could be related to a volume-sensitive anion channel (reviewed in Strange et al., 1996). Absence of sensitivity of the non-saturable uptake to hyperosmolarity, lack of Na^+ and Cl^- dependence, absence of inhibition by taurine analogues prove that the non saturable system is not a putative volume-activated "taurine channel" but a diffusion process. This agrees with the report of Christensen and Liang (1966) showing that the non saturable uptake of taurine in rat jejunum is mainly diffusion. Previous studies have shown the presence of a significant diffusion process in mammalian intestine (Buffoni et al., 1978; Kim, 1983; Barnard et al., 1988; Moyer et al., 1988; Wolffram et al., 1991; Sharafuddin and Nassar, 1993; Barada et al., 1997). By contrast, the diffusion process found in Caco-2 (Satsu et al., 1997) or chemosensitive LoVo cells was less important. This could be related to the species (human versus non-human) or to the fact that the studied cell lines were tumoral.

We have determined taurine basal efflux as the amount of taurine going out of the cells in the same conditions as those used during uptake measurement. At present, nothing is known about the mechanism(s) of basal (non-stimulated) taurine efflux: is there one (LoVo S) or more (LoVo Dox) system? The taurine efflux in the two LoVo S cells was negligible when the external taurine concentration was low, as previously observed in normal rat glial cells (Lleu and Rebel, 1989) or neuroblastoma x glioma hybrids (Kürzinger and Hamprecht, 1981). Increase of external taurine concentration hardly affected the taurine efflux in LoVo S fusoid cells but increased it in LoVo S small cells to a level similar to those previously observed in heart (Grosso et al., 1978) or glioma cells (Schrier and Thompson, 1974). The basal efflux of LoVo Dox was high and only slightly affected by external taurine. This high efflux is probably not related to the MDR status of LoVo Dox cells. Indeed, Galietta et al. (1996) have found no difference in the basal taurine efflux (isoosmotic efflux) of MDR and non-MDR human tracheal 9HTEo- cells. Moreover, an opposite result was observed with KB cell lines, chemosensitive cells having a high taurine efflux and MDR cells a low efflux (Wersinger et al., in preparation). However, we can not exclude that the efflux mechanism is cell specific, being in LoVo cells affected by the presence of P-glycoprotein.

An important difference between LoVo Dox and its chemosensitive counterparts LoVo S is related to the overexpression of P-gp and few other proteins in the MDR cell plasma membrane (Grandi et al., 1986). Stimulation or inhibition of P-glycoprotein-mediated drug transport by growing cells in a medium supplemented with doxorubicin or verapamil, did not affect taurine uptake. P-glycoprotein can make in some MDR cells up to 1% of the total plasma membrane proteins (Ambudkar et al., 1992). However, our results demonstrate that alteration in plasma membrane composition related to the overexpression of P-gp did not alter the main properties of the taurine transport systems, only modifying the kinetic parameters of the taurine high and low affinity uptake systems. These results suggest a reduction of the

maximal velocity of the two transport systems and a decreased affinity for taurine of the low affinity transport system in LoVo Dox cells. Comparing the chemosensitive KB-3-1 cells with the MDR KB-V-1 cells overexpressing P-gp, we also observed a change of high and low affinity taurine uptake kinetics in MDR cells (Wersinger et al., in preparation). Similarly, a decreased activity and number of glucose transporters GLUT-1 was recently observed in KB MDR cells (Bentley et al., 1996). A decrease of Vmax is related to a reduced number of transporters in the plasma membrane. Overexpression of the multidrug transporter could either render some taurine transporters cryptic, inhibit the synthesis of the transporters at a transcriptional level or at a posttranscriptional level (phosphorylation), or decrease the incorporation of the transporters into the LoVo Dox plasma membrane by spatial hindrance. A change in cell lipids has been previously shown to modify the kinetic parameters of the high affinity taurine uptake system (Balcar et al., 1980; Yorek et al., 1984). Analysis of the lipids composition of MDR cells and their chemosensitive counterparts have shown only minor changes (summarised in Alon et al., 1991 and Callaghan et al., 1992). Though the fatty acid composition of MDR and non-MDR cells is very close, a decrease in the acyl chain mobility is observed in MDR cells (Callaghan et al., 1992), which could result from the insertion of new proteins or increased levels of some proteins in the plasma membrane. Though these observations do not exclude completely a role of lipids in the modulation of taurine (or glucose) transport in MDR cells, they show that the plasma membrane protein distribution has surely a role in this modulation.

Although the Vmax of high and low affinity transport systems was reduced and the Km of the low affinity system was increased in LoVo MDR cells, taurine uptake was greater in LoVo Dox cells compared to the two LoVo S lines. This apparent contradiction could perhaps be explained by the greater diffusion component in LoVo Dox cells compared to the two LoVo S strains. Taurine diffusion is an important influx component for the intestinal uptake of dietary taurine, where high affinity uptake notably decreases or disappears with ageing (Moyer et al., 1988; Wolffram et al., 1991). However, we have presently no idea about the physiological role of diffusion in vivo. We could expect that this uptake system would be important when high amounts of taurine (1 g or more) are given to a patient.

A difference between the previously studied Caco-2 and HT29 cells and our LoVo cells is that the first two lines were derived from a primary colon carcinoma while LoVo line was obtained from a supraclavicular metastasis of a colon carcinoma. Our results show that a similar taurine uptake system (at least for the high affinity system) exists in the Caco 2, HT29 and LoVo cell lines. Tumour cell metastasis is partially related to changes in the plasma membrane composition. This last modification apparently does not alter the main characteristics of a transporter such as the taurine transporter.

Jhiang et al. (1993) using tumour thyroid cells have hypothesised that the greater the cell growth rate is, the higher is the taurine uptake. Results obtained with LoVo cells do not agree with this hypothesis. In fact LoVo Dox cells, which proliferate significantly slower than the two LoVo S lines have

greater taurine uptake levels than the two LoVo S cells. There was also no direct correlation between the uptake levels of LoVo Dox cells and their proliferating status. The high affinity uptake of the two LoVo S cells decreased when cultures became confluent. The absence of variation in LoVo Dox was probably related to the inability of these cells to establish a true confluence. High affinity uptake also varied with the culture density in HT29 cells, decreasing at confluence (Tiruppathi et al., 1992). However, in HT29 cells, the protein level per dish notably increases after the cultures become confluent (Fig. 8 in Tiruppathi et al., 1992). Either the confluence is not associated with a growth arrest or the amount of protein/cell changes with time. Under these last conditions, since protein varied, it cannot be used as a reference for uptake measurement as reported in this last paper.

Acknowledgements

We thank Dr L. Capolongo from the Pharmacia & Upjohn for providing us with the LoVo and LoVo/DX cell lines.

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Received May 19, 1999