Amino Acids (2004) 26: 183–195
DOI 10.1007/s00726-003-0035-7

Amino Acids
Printed in Austria

Effects of culture conditions on taurine uptake by various variants of human endometrial carcinoma cells in culture

S. Deret, J. Voegelin, I. H. C. Lelong-Rebel, and **G. Rebel**

UPR 9003 CNRS, Institut de Recherche contre les Cancers de l'Appareil Digestif, Hôpital Civil, Strasbourg, France

Received October 3, 2002
Accepted April 30, 2003
Published online September 17, 2003;

Summary. The general properties of the taurine uptake in human endometrial tumoral Ishikawa cells were similar to those usually found in other tissues. Uptake was notably affected by the oxygen pressure, being higher at the physiological pO_2 of the endometrium (40 mm Hg, equivalent to 5% O_2) compared to that used under standard experimental culture conditions (160 mm Hg or 20% O_2). Uptake of taurine was also density-dependent in Ishikawa cells and was significantly decreased at confluence. Uptake regulation by PKC driven phosphorylation occurs only in growing cells and not in resting cells. The taurine uptake of three Ishikawa cell lines was very different. The taurine uptake of one of the cell lines was affected by estradiol, probably through a non-genomic pathway, whereas tamoxifen had no effect in all cell lines.

Keywords: Taurine uptake – Estradiol – Tamoxifen – Oxygen pressure – Ishikawa cell line

Introduction

Taurine, an ubiquitous aminoacid in mammalian tissues, is involved in cellular homeostasis regulation through its effect on osmotic regulation, in protection against some effects of oxidative stress, in detoxification and membrane stabilisation (Huxtable, 1992). Because of its important physiological roles, taurine uptake has been studied in numerous mammalian tissues such as brain, lung, liver, intestine, kidney and placenta. To the best of our knowledge, few studies have been devoted to taurine uptake regulation in well-known hormone-dependent tissues. A high affinity taurine uptake has been found in mammary glands (Shennan and Mc Neilie, 1994; Shennan, 1995; Bryson et al., 2001), in placenta (Miyamoto et al., 1988; Karl and Fisher, 1990; Hibbard et al., 1990) and in placental choriocarcinoma JAR cells (Kulanthaivel et al., 1991; Jayanthi et al., 1995). Endometrium is also a tissue the physiology of which is also largely dependent on estrogens. However, by contrast to mammary gland and placenta nothing is known about the possible existence of a taurine uptake system in endometrial epithelial cells. We addressed the study of taurine uptake and its main characteristics, using three variants of the Ishikawa cell line. Ishikawa cells are derived from a human endometrial carcinoma and constitute the most popular cell model used for studies on hormone-dependent mechanisms in endometrium. (Nishida et al., 1985).

In vivo, the physiological oxygen tension (pO_2) around endometrium is close to 40 mm Hg (5% O_2) (Yedwab et al., 1976; Rodesch et al., 1992). Moreover, the *in vivo* oxygen tension of all the solid tumour type tested is low, most of the cells being at a pO_2 of 40 mm Hg or less (Vaupel et al., 1989; Helmlinger et al., 1997; Brown and Giacca, 1998; Adam et al., 1999). Tumours of the uterus are also characterised by a very low pO_2 (Vaupel et al., 1989; Höckel et al., 1991; Lyng et al., 1996; Sunfør et al., 1998; Adam et al., 1999; Aquino-Parsons et al., 2000). It is known that hypoxia modulates the activity of numerous genes (Guillemin and Krasnow, 1997; Semenza, 1998). As no studies have addressed the issue of how oxygen pressure might affect a physiological transport, we have screened all the properties of taurine uptake on cells grown either at 5% O_2 – the oxygen level found *in vivo* – or at 20% O_2, the oxygen pressure always used under standard culture conditions e.g. in [5% CO_2-air] incubator atmosphere.

Material and methods

Chemicals

[^{3}H]-taurine (29 Ci/mmol) was from DuPont-NEN (NET 541). Unlabeled taurine was from Janssen Chimica. HEPES (monosodic salt) and other Good buffers, taurine analogues, 17β-estradiol, tamoxifen, PKC activator

12-O-tetradecanoylphorbol-13-acetate (TPA), not biologically active 4α-phorbol 12,13 didecanoate (4αPDD), PKC inhibitor hexadecyl phosphocholine (HePC), were from Sigma. All other chemicals were of analytical grade.

Cell cultures

Three Ishikawa cell lines from different laboratories were used. Ishikawa-S line was obtained from the Institute of Genetic and Molecular Biology – Strasbourg, France (IGBMC, Prof. P. Chambon). Ishikawa-B line was obtained from the Dept. of Biochemistry and of Molecular Biology of the University of Besançon – France (Prof. M. Jouvenot) and Ishikawa-M line was obtained from INSERM U418-Montpellier – France (Prof. H. Rochefort). Detailed characteristics of the specificities of the three cell lines were reported elsewhere (Deret et al., in preparation). In summary, when received in our laboratory the growth of Ishikawa-S was unaffected by estradiol (E2) whereas cell growth was moderately increased by E2 for Ishikawa-B cells and strongly for Ishikawa-M cells. Alkaline phosphatase activity was notably stimulated by estradiol in all cell lines whereas it was also increased by tamoxifen in Ishikawa-M cells but inhibited by tamoxifen in Ishikawa-B and Ishikawa-S cells. Cells were routinely grown in T25 vented flasks (Corning) in low glucose, phenol red containing DMEM (Sigma, D5523) supplemented with 2 g/l sodium bicarbonate and 50 UI/ml penicillin/50 μg/ml streptomycin (DMEM:PR+). Cultures were supplemented with 5% heat inactivated fetal calf serum (FCS) freshly received from the manufactor (Institut Jacques Boy, Reims, France). Cultures were replicated with trypsin-EDTA pH 8 then grown in 20% O_2 atmosphere or in 5% O_2 atmosphere in a 3-gas incubator. When estrogen or antiestrogen were tested, cultured cells were transferred for 2 days before the beginning of the experiments in phenol red free, low glucose DMEM (Sigma, D2902) supplemented with 2 g/l sodium bicarbonate and antibiotics (DMEM:PR−) and with 5% heat inactivated newborn calf serum (NCS, Institut J. Boy). Estradiol and tamoxifen were added to the culture as an ethanolic solution, the ethanol concentration in the culture medium remaining lower than 0.5%. Similar amounts of ethanol were added to control cells. The osmolarity of media was in the physiological range of 285–290 mOsm (Waymouth, 1970).

For experiments, cultures were prepared according the following procedure: Ishikawa cells were trypsinized using pH 8 trypsin-EDTA solution in order to obtain a suspension of isolated cells e.g. more than 90% single cells devoid of large clumps. Cells were then plated in 3 cm diameter Petri dishes (Corning 430165) in 2 ml culture medium and the seeding density was adjusted in order to achieve the required cell density for testing after 3–4 days of growth and under the chosen pO_2. Phase contrast microscopy observations showed that under these conditions the size and density of cell patches was reproducible and similar in the various sets of experiments (see discussion for the importance and relevance of this particular protocol).

Two types of experimental sets were made. In a first approach, each cell line was tested alone. Thereafter, the three cell lines were compared in parallel experiments with cells at similar density and cultured under the same conditions.

Cell lines were routinely tested using Hoechst 33258 stain and found to be free of Mycoplasma.

Taurine uptake

Taurine uptake was measured on cells cultured in 3 cm diameter Petri dishes according to Lelong and Rebel (1998b). Briefly, treated cells were washed twice with CO_2-independent medium (CIM, Gibco) and pre-incubated for 5 min in 3 ml of CIM in a rotatory water bath at 37°C. Then, 100 μl of CIM containing 0.4 μCi [^{3}H]-taurine and 1.33 μM of unlabeled taurine were added to the pre-incubation medium and cells were incubated for 10 min. After removal of the medium, cells were washed three times with ice-cold 0.9% NaCl and subsequently frozen in their dishes after removing the saline. Thawing and lysis of the cells was performed by adding of 1 ml 0.1 N NaOH and incubating for 1 h at 37°C. Samples were homogenised by repetitive pipetting and aliquots were withdrawn for protein assay according to Lowry et al. (1951) and for radioactivity measurements by scintillation counting in Aquasol (Packard, Nef 934-G). When sodium or chlorine dependence of taurine uptake was determined, pre-incubation and incubation of control cells were performed in Krebs-Ringer buffer (KR buffer: 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); 5 mM D-glucose, pH 7.4. Na^+-dependence of taurine uptake was checked by replacing NaCl and NaH_2PO_4 by LiCl and by KH_2PO_4 (mole per mole, respectively) in the KR buffer formula. For chlorine-dependence determination, NaCl, KCl and $CaCl_2$ were replaced by identical molarity of sodium-, potassium- and calcium-gluconate respectively. Osmolarities of KR, Na-free KR and Cl-free KR buffers were similar (290 mOsm). High and low affinity uptakes were quantified incubating cells with either 1.33 μM or 200 μM taurine (Wersinger et al., 2000). For diffusion, cells were incubated in Na-free KR buffer.

Taurine efflux measurement

Cells grown on 3 cm diameter Petri dishes were loaded with 0.4 μCi [^{3}H]-taurine in the culture medium for 2 hours at 37°C in incubator. Cells were then treated as follows: a first set of four dishes was washed three times with ice cold 0.9% NaCl and was immediately frozen (control); other sets of dishes were washed three times with 3 ml of CIM at 37°C, and incubated for 10 min in 3 ml of Krebs-Ringer buffer containing either 1.33 μM or 200 μM unlabeled taurine or in Na-free KR buffer with 1.33 μM unlabeled taurine in a giratory water bath at 37°C. After 10 min incubation, medium was removed and cells were washed three times with ice-cold 0.9% NaCl and frozen. Cells were homogenised in 1 ml of 0.1 N NaOH by repetitive pipetting. Aliquots were withdrawn for protein and radioactivity determination. The efflux data were calculated as the difference between the radioactivity per mg of protein in control cells versus incubated cells. Efflux was expressed in % of the taurine content in control cells.

Effect of oxygen pressure

Since oxygen pressure affects notably the growth kinetic of the three Ishikawa cell lines (Deret et al., in preparation), the following procedure was used when data on cells grown at 5% and 20% O_2, were compared. Cells were plated in Petri dishes at identical cell density and were subsequently grown at 20% O_2 until the required density was achieved. Then cells were fed with fresh culture medium supplemented with 2% serum and moved to the appropriate incubator with either 5% or 20% O_2, and growth was allowed to proceed for two days before measuring taurine uptake. Under these conditions, cell proliferation continued at a low rate thus avoiding notable differences in cell density between cultures maintained at 5% or 20% O_2.

Proteins were determined according to Lowry et al. (1951). Media osmolarity was measured with a Roebling osmometer. Each result was the mean of at least four different values ± standard deviation. The statistical significance of the difference between two means was evaluated by Student's independent two-tailed *t-test*. $P < 0.05$ was considered to denote statistical significance.

Results

General properties of taurine uptake in Ishikawa cell lines

Though the uptake capacity of the three Ishikawa cell lines differed notably, the overall pattern of their taurine

uptake systems were similar (Na^+, Cl^- dependence, specificity ....). The 3 cell lines were found very similar when results were expressed as per cent of controls. As the uptake was notably greater in low density than in confluent cultures, all the basic properties of taurine uptake were determined on growing cells. Moreover, these properties were checked for cell cultures maintained at 40 mm Hg of O_2 (5% O_2) atmosphere, a pO_2 in the physiological range of the endometrium pO_2. As protein/DNA ration of Ishikawa cells was similar in growing and in confluent cells, results were always referred to protein concentration.

Cell incubation conditions

To avoid the use of the so-called ''physiological buffers'', the composition of which being far from that of physiological media, cells were incubated in CIM medium for taurine uptake determination as previously described (Lelong and Rebel, 1998b). Under these conditions, taurine uptake was linear for at least 40 minutes. As previously reported by Lelong and Rebel (1998b) for glial cells, uptake of taurine was lower in cells incubated in CIM compared to cells incubated in Krebs-Ringer buffer (Table 1).

Characteristics of taurine uptake systems

Figure 1 shows that uptake increased with increasing taurine concentrations. The Eadie-Hoffstee plot shows the presence of a high and a low affinity uptake system. The presence of a small Na^+-independent uptake indicates the probable existence of a diffusion system. As the kinetic parameters of the Ishikawa cells were in the same range as that previously measured for LoVo colon carcinoma cells (Wersinger et al., 2000) we used the same concentrations of unlabeled taurine to determine high and low affinity uptake. This also facilitates the comparison of taurine uptake behavior in tumorous cell lines differing in tissue origin.

The uptake level was greatly affected by cell density being the lowest at confluence (Table 3). Decrease of the uptake occurred progressively as cell density increased. Comparison of the 3 cell lines grown in identical conditions shows that the high affinity level differs greatly between the 3 lines being the highest in Ishikawa-S and the lowest in Ishikawa-B (Table 4). Though it was difficult

Table 1. Modulation of taurine uptake by ions and Good buffers

Culture medium	Incubation medium	Taurine uptake ± SD pmol/min/mg protein	% uptake
DMEM	KR	132.0 ± 2.9	100
DMEM	KR:Na−	2.1 ± 0.4	2
DMEM	KR:Cl−	1.9 ± 0.1	2
DMEM	CIM	101.1 ± 9.3	77
DMEM	KR	93.3 ± 5.0	100
DMEM	KR:HEPES	107.1 ± 4.9	115
DMEM	KR:ACES	98.1 ± 3.5	105

Ishikawa cells were grown up to half confluence in (DMEM:PR+) medium with 5% FCS at 5% O_2. Uptake was determined in cells incubated either in Krebs-Ringer (KR) or in KR without Na^+ (KR:Na−) or without Cl^- (KR:Cl−), or in KR supplemented with 20 mM of HEPES or of ACES. Osmolarity of KR, KR:Na−, KR:Cl−, KR:HEPES, KR:ACES was between 274 and 284 mOsmol. Uptake variations of 20% or more are significant at $p < 0.05$

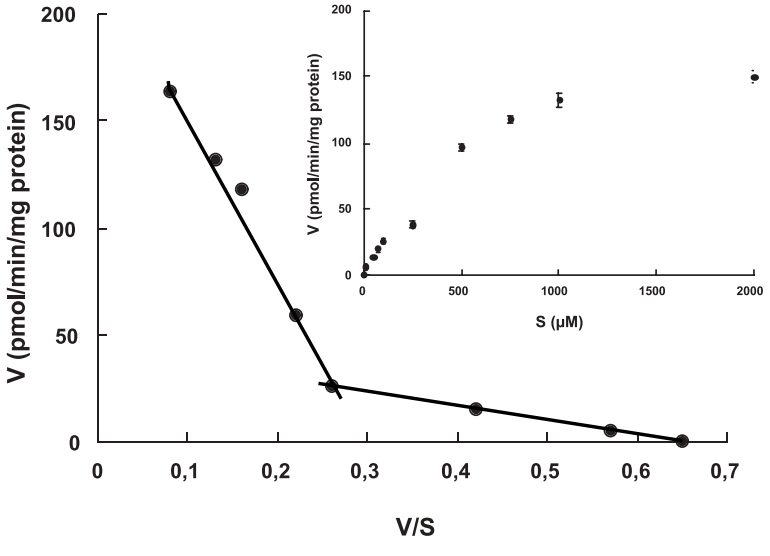

Fig. 1. Kinetic analysis of taurine uptake. Ishikawa-S cells were grown in (DMEM:PR+) with FCS at 5% O_2 until 1/4 confluence. Initial rates were determined with 10 min preincubation in CIM containing increasing concentrations of unlabelled taurine (0 up to 2 mM) followed by 10 min incubation after addition of labelled taurine. Final concentration of radiolabeled taurine was maintained constant at 0.4 μCi (5nM [^{3}H]-taurine) for each concentration of unlabeled taurine. Results are given as Eadie-Hoffstee plot. Inset: taurine uptake as a function of taurine concentration. *V:* taurine uptake ± SEM (pmol/min/mg protein); *S:* taurine concentration (μM)

Table 2. Specificity of taurine uptake

Compounds tested	Taurine uptake ± SD pmol/min/mg protein	% control
–	99.2 ± 5.1	100
Taurine 0.05 mM	32.7 ± 0.5	33*
Taurine 0.1 mM	24.8 ± 0.4	25*
Taurine 1 mM	4.6 ± 0.3	5*
β-alanine 1 mM	8.6 ± 1.4	9*
GES 1 mM	1.7 ± 0.2	2*
α-alanine 1 mM	108.0 ± 5.7	109

Half confluent cell cultures were grown in (DMEM:PR+) as in Table 1. Taurine uptake was measured in cells incubated in CIM supplemented or not with taurine at 0.1, 0.5, and 1 mM or with taurine analogues at 1 mM. Differences from control cells are significant at $^*p < 0.01$

Table 3. Effect of cell density on taurine uptake

Cell line	Cell density	μg protein/ dish	Taurine uptake ± SD pmol/min/mg protein
Ishikawa-M	Low	52	253.4 ± 10.2
	Half confluence	146	144.7 ± 3.8
	Confluence	360	21.0 ± 2.1
Ishikawa-B	Low	114	60.9 ± 2.7
	Confluence	524	5.4 ± 1.1
Ishikawa-S	Very low	21	1011.0 ± 51.7
	Half confluence	181	513.4 ± 14.1
	Confluence	482	273.1 ± 24.7

Cultures were grown at 5% O_2 in (DMEM:PR+) supplemented with FCS until the required density

to quantitate the low affinity precisely, due to its partial overlapping with the high affinity uptake, it is quite certain that the same difference exists between the 3 cell lines. Considering the diffusion, it always remained very low (Table 4). The difference between the respective taurine uptake of the cell lines was not related to taurine efflux. Whereas efflux of taurine was absent or very low (less than 1% of the taurine taken up) in S and in M cells, only confluent B cells show a statistically significant efflux in high affinity uptake conditions (Table 4).

Properties of the high affinity taurine uptake system

A sodium and chlorine-uptake dependence, a characteristic of taurine transport (Huxtable, 1992), was also found in Ishikawa cells (Table 1). Taurine uptake inhibition by taurine, GES or β-alanine showed that this uptake system was specific for β-aminoacids. Under the same conditions, α-alanine did not affect the uptake (Table 2). HEPES, an inhibitor of taurine uptake system in nervous cells (Lleu and Rebel, 1989) or ACES did not significantly affect the uptake.

Effect of phenol red and serum type on taurine uptake

To avoid a possible interference by phenol red (Berthois et al., 1986), hormone-dependence of taurine uptake was studied in cells grown in a phenol red free medium supplemented with NCS. Presence or absence of phenol red in the culture medium did not affect the taurine uptake (uptake in cells grown in DMEM:PR− was 95% of the uptake of cells in DMEM:PR+). Similar results were also obtained when uptake was determined in cells grown in the presence of the same concentration of fetal calf serum or newborn calf serum (uptake in medium supplemented with 5% NCS represented 104% of that determined on cells grown in 5% FCS).

Table 4. Cellular level of taurine uptake systems and taurine efflux

Cell line	Culture conditions	Taurine uptake pmol/min/mg protein			Taurine efflux % taurine effluxed under the condition used for measuring		
		high affinity	low affinity	diffusion	high affinity	low affinity	diffusion
Ishikawa-S	Confluent	145 ± 8	2747 ± 170	6 ± 1	0	0	0
	Growing	1078 ± 30	8965 ± 540	16 ± 3	0	1	0
Ishikawa-M	Confluent	76 ± 10	1322 ± 120	11 ± 1	1	0	0
	Growing	383 ± 40	6148 ± 256	22 ± 1	0	0	0
Ishikawa-B	Confluent	17 ± 1	109 ± 20	9 ± 1	38	9	2
	Growing	71 ± 4	675 ± 21	8 ± 1	0	0	0

Cell cultures were grown as indicated in "Material and methods" in (DMEM:PR+) with FCS at 5% O_2 until the required density. Uptake was determined by incubating cells in KR, in presence of either 1.3 μM (high affinity uptake) or 200 μM (low affinity) unlabeled taurine or in KR without Na^+ (diffusion). Efflux was determined as described in "Material and methods"

Table 5. Effect of O_2 level on taurine uptake of cultured cells

Cell line	Density	% oxygen	Taurine uptake ± SD pmol/min/mg protein	Percentage		
				(a)	(b)	(c)
Ishikawa-M	Low	5	253.8 ± 8.0	100	100	
		20	67.3 ± 3.0	26		100
	Confluent	5	20.7 ± 2.0	100	8	
		20	17.7 ± 2.0	85		26
Ishikawa-B	Low	5	60.6 ± 1.8	100	100	
		20	41.1 ± 0.5	68		100
	Confluent	5	5.3 ± 0.5	100	8	
		20	6.4 ± 1.1	120		15
Ishikawa-S	Low	5	675.5 ± 18.8	100	100	
		20	469.5 ± 5.7	69		100
	Confluent	5	286.3 ± 14.7	100	42	
		20	249.9 ± 26.3	87		53

Ishikawa cells were grown in (DMEM:PR+) with FCS for at least two days at the tested O_2 concentrations. Percentages: (a) uptake in cells grown at 5% O_2 versus cells grown at 20% O_2; (b) uptake in confluent versus low-density culture at 5% O_2; (c) uptake in confluent versus low-density culture at 20% O_2. Difference of 20% or more are significant

Effect of pO_2

For low density growing cells taurine uptake was reduced when cultured at 20% O_2 compared to 5% O_2 (Table 5). This was more noticeable with Ishikawa-M cells than with Ishikawa-S or with -B cells. However, when uptake was measured on confluent cells, pO_2 had little effect on taurine uptake. The lack of action of oxygen on taurine uptake in confluent Ishikawa-B cells is certainly related to the very low level of the uptake.

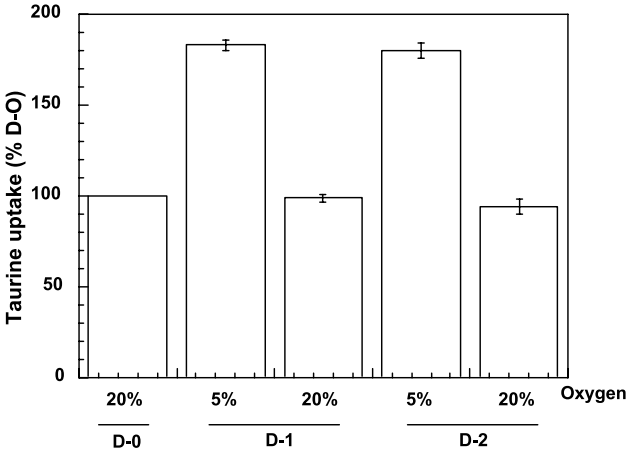

Fig. 2. Effect of pO_2 on taurine uptake. 1/4 confluent Ishikawa M cells were grown in (DMEM:PR+) with 5% FCS at 20% O_2. Day-0 cells (D-0) were changed to (DMEM:PR+) with 2% FCS. Half of the Petri dishes were switched to 5% O_2 or remained at 20% O_2 (J1, J2 cells). Taurine uptake was determined on D-0, D-1 and D-2 respectively. Similar results were obtained with Ishikawa-B and Ishikawa-S cells

For convenience, stock cultures were maintained at 20% O_2 [e.g. air/5% CO_2 in a standard cell culture incubator]. When required for the experiments, cultures were replated and kept at 20% O_2 or switched to 5% O_2 [e.g. 5% O_2/5% CO_2] for several days (minimum 2 days) prior to uptake determination. Figure 2 shows that after 24 hours at 5% O_2, uptake levels reached a value that did not change further.

Table 6. Effect of TPA concentration on taurine uptake

Medium supplementation	Taurine uptake (pmol/min/mg protein) in cells grown at	
	5% O_2	20% O_2
none	95.4 ± 9.9 (100)	73.8 ± 3.8 (100)
TPA 1 nM	86.5 ± 6.4 (90)	68.3 ± 7.4 (92)
TPA 10 nM	70.4 ± 7.8 (74)*	60.6 ± 7.8 (82)
TPA 100 nM	57.8 ± 5.5 (60)*	53.6 ± 4.0 (72)*
TPA 1 μM	48.9 ± 6.1 (51)**	43.3 ± 4.0 (58)**
TPA 10 μM	49.7 ± 5.2 (52)**	42.0 ± 2.5 (57)**
4αPDD 1 μM	83.6 ± 3.0 (88)	
HeDC	88.7 ± 7.3 (93)	
TPA + HeDC	91.5 ± 7.3 (96)	

Ishikawa-M cells were grown in (DMEM:PR+) with FCS at 5% or 20% O_2. Half-confluent cultures were treated with increasing concentrations of TPA or with 1 μM 4α-phorbol 12,13 didecanoate (4αPDD) for 150 minutes. Another set of dishes was treated either with 1 μM hexadecylphosphocholine (HePC) for 180 minutes or with 1 μM HePC for 180 minutes together with 1 μM TPA for the last 150 min. Percent of control cells are indicated into brackets. Results statistically different from control (untreated) cells: $^{*}p<0.05$; $^{**}p<0.01$

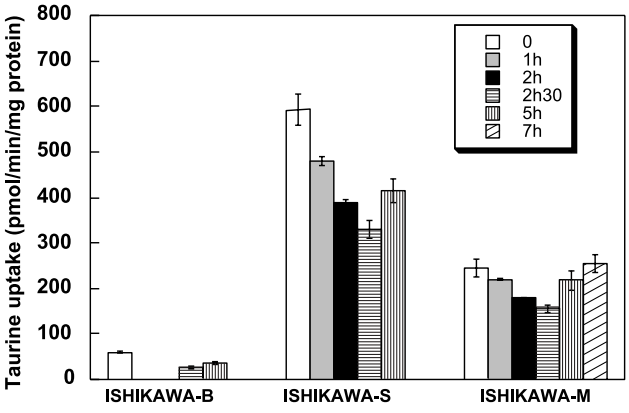

Fig. 3. Reversibility of the TPA induced taurine uptake inhibition. Ishikawa cells grown in (DMEM:PR+) with FCS. 1/4 confluent cells were treated with 1 μM TPA for increasing time and taurine uptake was determined thereafter

Modulation of taurine uptake by protein kinase modulators

The PKC activator tetradecanoyl-phorbol-acetate (TPA) inhibited taurine uptake in the three cell lines. The TPA effect was independent of the type of serum used (FCS or NCS) and of the presence or the absence of phenol red (not shown). TPA inhibited uptake similarly in cells grown either at 20% O_2 or 5% O_2 (Table 6). Under the same conditions 4α-phorbol 12,13 didecanoate was inactive. Maximal inhibition was obtained by incubating the cells with 1 μM TPA for 150 min (Fig. 3). Reversal of the inhibitory effect occurred immediately after (Fig. 3). A quite similar TPA effect was observed in all cell lines (Fig. 3, Table 7). The less the cultures were growing, the lower was the inhibition of uptake, TPA being without effect on confluent cultures as well as on non growing low density cultures (Tables 7 and 8). The PKC inhibitor hexadecylphosphocholine (HDC) weakly inhibited the uptake and reversed completely the TPA inhibition (Table 6).

The calmodulin inhibitor W13 did not change the uptake at concentration of 1 nM–10 μM over 30–120 minutes. Similarly, increasing cell cAMP level by treatment with either SpcAMP (1–5 μM) or with dbcAMP (1 mM), had no effect on taurine uptake, thus excluding involvement of protein kinase A (PKA) (data not shown).

Effect of estradiol and of tamoxifen

Table 9 shows that low-density culture taurine uptake was stimulated by estradiol only in Ishikawa-M cells. This stimulation was greater for cells grown at 5% O_2 for two days in DMEM:PR− supplemented with NCS, compared to that of the same cells grown at 20% O_2. However, E2 concentrations of 10 nM to 10 μM produced a similar stimulation. Under identical culture conditions, tamoxifen (100 nM–1 μM) did not significantly affect taurine uptake in the M and S cell lines cultured at 5% O_2 or 20% O_2 (Table 9). A slight inhibition was observed in B cells.

Table 7. Effect of cell density on TPA inhibition of taurine uptake

Cell line	Cell density	TPA	Taurine uptake ± SD pmol/min/mg protein	Percentage
Ishikawa-M	Confluent	−	21.0 ± 2.1	100
		+	21.5 ± 1.8	100
	1/3 confluent	−	144.7 ± 3.8	100
		+	79.1 ± 6.6	55
	Low	−	253.4 ± 10.2	100
		+	135.3 ± 8.0	61
Ishikawa-B	Confluent	−	5.4 ± 1.1	100
		+	6.0 ± 1.5	111
	Low	−	60.9 ± 2.7	100
		+	29.3 ± 3.3	48
Ishikawa-S	Confluent	−	273.1 ± 24.7	100
		+	260.3 ± 13.3	95
	Low	−	675.0 ± 28.8	100
		+	331.9 ± 19.8	49

Cells were grown in (DMEM:PR+) with FCS until the required density. TPA (1 μM) was added to the culture medium for 150 min before uptake determination. Results statistically different from non-treated culture: $^{*}p < 0.01$

Table 8. Effect of cell growth on TPA inhibition of taurine uptake

Culture density	Protein (mg/dish)	TPA	Taurine uptake	
			pmol/min/mg protein	%
Confluence	360	–	48 ± 1	100
	350	+	42 ± 1	88
Near confluence	313	–	93 ± 3	100
	315	+	77 ± 3	82
Half confluence with isolated cells	132 ± 7	–	167 ± 10	100
	127 ± 2	+	117 ± 6	70*
Half confluence with patches of cells	109 ± 3	–	152 ± 9	100
	110 ± 7	+	149 ± 18	98

Cells were seeded at various densities in order to obtain the required densities simultaneously. Cells were grown at 20% O_2 in (DMEM:PR+) with 5% FCS excepted for the half confluent isolated cells which were grown in presence of 2% FCS. Two days before uptake determination, cultures were transferred at 5% O_2. Medium was changed to (DMEM:PR+) either with 5% FCS for ''confluent'' and ''nearly confluent'' sets, or with 2% FCS for ''half-confluent'' cells. TPA (1 μM) was added to the culture 150 min before uptake determination. Results statistically different from non treated cultures: $^{*}p < 0.05$

Table 9. Effect of estradiol and of tamoxifen on taurine uptake

Cell line	Culture medium supplementation	Oxygen level (%)	Control uptake pmol/min/mg protein	Taurine uptake (% control cells) in cells cultured in presence of nM E2 or tamoxifen			
				1 nM	10 nM	100 nM	1000 nM
Ishikawa-M	E2	5	195	137*	184**	197**	170**
		20	206	122*	151**	157**	160**
	Tamoxifen	5	267	91	88	90	81
		20	262	96	91	95	88
Ishikawa-B	E2	5	33	91	97	108	97
		20	42	96	102	95	107
	Tamoxifen	5	46	71*	80	75*	73*
		20	50	70*	75*	75*	75*
Ishikawa-S	E2	5	445	91	93	89	88
		20	442	90	93	107	102
	Tamoxifen	5	444	–	102	106	99
		20	419	–	100	95	93

Cells were grown in (DMEM:PR−) with 5% NCS until half confluent. Estradiol (E2) or tamoxifen were added to the culture medium for the last two days. Statistical difference from control cells (untreated) at $^{*}p < 0.05$, $^{**}p < 0.01$

Taurine uptake in Ishikawa-M cells was similarly affected when tamoxifen was tested either as an anti-estrogen (e.g. cell cultures performed for two days in DMEM:RP− +2% NCS, $+10^{-7}$ M tamoxifen) or as a PKC inhibitor (e.g. cell cultures performed for two days in DMEM:PR+, $2 \cdot 10^{-5}$ M tamoxifen added for the last 3 hours prior to uptake measurement (Gundimeda et al., 1996) (compare Tables 9 and 10). TPA inhibited taurine uptake similarly in Ishikawa-M cells whether they were treated or not either with E2 or with tamoxifen (Table 10). Whatever the concentrations, estradiol or tamoxifen had no effect on uptake when added for 10 min to the incubation medium of the three cell lines during the taurine uptake determination.

Table 10. Effect of TPA on estradiol or tamoxifen treated cells

Culture medium supplementation		Taurine uptake			
		pmol/min/mg protein	%		
			(a)	(b)	(c)
None (control)		264 ± 26	100		
TPA	1 μM–2 h	161 ± 11	61*		
E2	100 μM–48 h	393 ± 2	149	100	
E2 + TPA	100 μM–48 h 1 μM–2 h	255 ± 8	96	65*	
Tamoxifen	100 μM–48 h	240 ± 18	91		100
Tamoxifen + TPA	100 μM–48 h 1 μM–2 h	167 ± 11	63*		61*
Tamoxifen	20 μM–3 h	239 ± 19	91		100
Tamoxifen + TPA	20 μM–3 h 1 μM–2 h	150 ± 14	59*		63*

Ishikawa-M cells were grown in (DMEM:PR−) with 5% NCS. 1/4 confluent cells were then cultured for two days with (DMEM:PR−) + 2% NCS supplemented or not with either estradiol (10^{-7} M) or tamoxifen (10^{-7} M) before taurine uptake determination. TPA (1 μM) was added or not for 2 h prior to taurine uptake determination. In another set of cultures, tamoxifen ($2 \cdot 10^{-5}$ M) was added 3 hours before uptake determination followed by TPA addition (1 μM) 2 hours prior to taurine uptake determination. Percentages: (a) uptake in percent of control cells (control); (b) uptake in percent of E2 treated cells; (c) uptake in percent of tamoxifen treated cells. Significant differences at $^{*}p < 0.01$

Discussion

In this study, cells were incubated in CO_2-independent medium (CIM) instead of the so-called ''physiological buffers'' that are buffered either with sodium bicarbonate or with HEPES (Lelong and Rebel, 1998a, b). Taurine uptake in cells incubated in CIM was lower than that measured with cells in Krebs-Ringer buffer. Similar results were observed for nervous cells (Lelong and Rebel, 1998b), and also for MDR and non-MDR KB and LoVo cells (Wersinger et al., 2000, 2001) (For discussion see Lelong and Rebel, 1998b). The presence of a high affinity and a low affinity taurine uptake is deduced from the plot of uptake versus taurine concentration. Although high affinity uptake is not the main taurine uptake system of Ishikawa cells, it is certainly the most important *in vivo* given that the low taurine concentration of extracellular media is certainly not higher than that of blood.

Taurine uptake systems from tumoral endometrium share the classical properties described for various tissues and cell lines such as Na^+, Cl^--dependence and specificity for β-aminoacids (Huxtable, 1992). HEPES and some other buffers synthesized by Good et al. (1966) inhibit taurine uptake in glial and glioma cells, in KB MDR and non-MDR cells (Lleu and Rebel, 1989, 1990; Petegnief et al., 1995; Lelong and Rebel, 1998b; Wersinger et al., 2001). In contrast, HEPES did not affect the uptake in Ishikawa cells. Similarly, ACES another N-ethylsulfonate derivative, which strongly inhibits taurine uptake in glial cells (Petegnief et al., 1995), had no effect in Ishikawa cells. HEPES has many side effects in mammalian cells some of which are cell specific (for the most important see Rebel et al., 1999). Our results show that the mechanism by which HEPES affects taurine uptake may differ between mammalian cells and is not simply related to a structural analogy between taurine and buffers described by Good.

To the best of our knowledge, very few studies have addressed changes of taurine uptake during the various stages of cell growth e.g. from low density growing cells up to confluent resting cells. In the three Ishikawa cell lines, taurine uptake decreases significantly when cultures reach confluence. We have previously reported a decrease of 50% in high affinity taurine uptake when human intestinal non-MDR LoVo carcinoma cells reach confluence (Wersinger et al., 2000). This was not observed with MDR-LoVo cells, probably due to their inability to produce non-growing confluent monolayer cultures. Our results agree with previously published data showing that the efficacy of various uptake systems such as amino acids (Weber et al., 1976; Borghetti et al., 1980; Petronini et al., 1982; Piedimonte et al., 1982, 1989) or glucose (Bradley and Culp, 1974) decreases at confluence in normal and

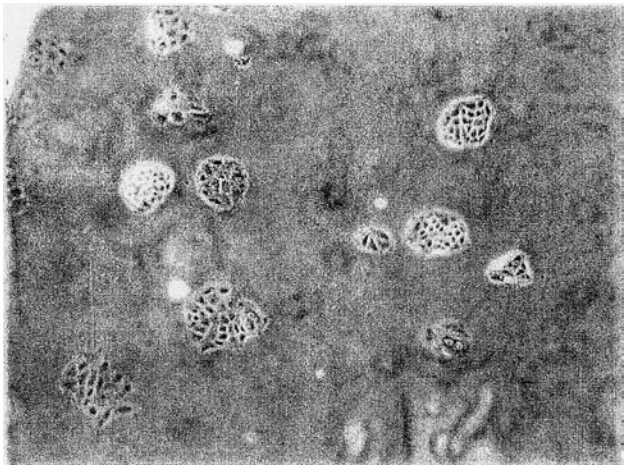

Fig. 4. Photomicrography of low density Ishikawa-M cells in culture. Stricktly similar pictures are obtained for Ishikawa-B and Ishikawa-S cells (× 60)

tumoral cells. Similarly, a decrease of binding for some receptors e.g. β-adrenergic receptors, has also been reported at cell confluence (Harden et al., 1979). Altogether, these observations suggest that upon reaching confluence e.g. a resting status for numerous cells, homeostasis maintenance is achieved by lowering the uptake of various components rather than by increasing their release.

Ishikawa cells do not grow as isolated cells. At mitosis, daughter cells do not separate, thus producing patches of increasing size with time (Fig. 4). Cells in the centre of the patch are non-growing whereas those in the periphery are dividing. This observation explains why taurine uptake in semi-confluent cultures is between the level found for low-density cultures and that found for confluent cultures (Table 3). Very low density cultures were characterised by a very high uptake level. In these cultures the majority of cells are either isolated or in contact with only 2 or 3 cells. With increasing density, the number of cells embedded in a patch (non-growing cells) increased whereas the taurine uptake concomitantly decreased. In confluent cultures all cells are completely in contact with others. Variation of the uptake with the cell density has an annoying consequence. Although the cell number of low-density cultures, in which patches are very small, does not seriously affect the uptake, this is not the case when the patches become larger. Significant differences in the uptake could be found between Petri dishes with similar DNA or protein content which differed in the size of their patches. Therefore, as detailed in ''Material and methods'', cell isolation and plating was performed with great care, to avoid a notable discrepancy in number and size of patches in the dishes. This procedure must also be used when the effect of TPA on taurine uptake has to be determined. Indeed, TPA inhibited taurine uptake only in growing cells (Table 8).

Usually cells are grown in an incubator with an atmosphere of air-CO_2 in which oxygen content represents 19–20%. Physiologically, this mimics a hyperoxic condition as the highest pO_2 found in human body reaches 16% (arterial pO_2, Hellkamp et al., 1991). The pO_2 of numerous tissues is notably lower, that of human endometrium being 5% (Yedwab et al., 1976; Rodesh et al., 1992). Moreover, most of the cells in a tumour are rather in a hypoxic, or even in an anoxic status (pO_2 0.1–5%). This is also the case for gynecological tumours (Vaupel et al., 1989; Höckel et al., 1991; Lyng et al., 1996; Sunfør et al., 1998; Adam et al., 1999; Aquino-Parsons et al., 2000). Comparison of taurine uptake at 20% and 5% oxygen shows that the uptake was notably higher at 5% oxygen. A shift from 20% oxygen to the ''physiological hypoxia'' was accompanied by an increase of taurine uptake in the absence of a noticeable change of efflux. Our results cannot be compared to those obtained by Franconi et al. (1985) and Schaffer et al. (2002), who observed an increase of taurine efflux or a decrease of cell taurine concentration in heart cells submitted to a metabolic hypoxia. The shift in the taurine uptake level when cultures were changed from 20% to 5% O_2 was very fast, being effective within one day. The rapidity of this increase paralleled the decrease of cell growth under the same conditions (Deret et al., in preparation).

The higher taurine uptake measured in cells at physiological pO_2 could be related to a protective mechanism against certain noxious consequences of hypoxia. Hypoxia induces an increase of reactive oxygen species (ROS), lower than the one found in hypoxia-reoxygenation but not negligible (Siraki et al., 2002). These free radicals induce lipid peroxidation (for a review, Rice-Evans and Burdon, 1993). Though we have not found any data on lipid peroxide levels in cultured tumoral cells, it has been shown that hypoxia increases the production of lipid peroxides and malondialdehyde, one of their degradation products (Yoshikawa et al., 1982; Minyailenko et al., 1990; Blasig et al., 1998; Serebrovskaya et al., 1999; Mankowskaya et al., 2000). Moreover, in several but not all cell types, hypoxia induces an increase in calcium influx (Buckler and Vaughan-Jones, 1994; Cornfield et al., 1993; Hu and Wang, 1994; Rose et al., 1993; Zhao et al., 1999). This could be due to the induction of an overload of intracellular calcium by ROS (Herson et al., 1999). Taurine has been shown to reduce the effect of

hypoxia both *in vivo* and *in vitro* (Schurr et al., 1987; Tseng et al., 1990; Kendler et al., 1989; Huxtable, 1992). Taurine on one hand regulates calcium movements (Liebowitz et al., 1989; Satoh, 1994) and antagonises calcium increase induced by hypoxia (Zhao et al., 1999). On the other hand, taurine reduces hypoxia driven lipid peroxidation (Mankovskaya et al., 2000) and inhibits the deleterious effects of reactive aldehydes by formation of Schiff bases (Ogasawara et al., 1993). Taurine could also protect cell membranes towards oxidative damage (Huxtable, 1992).

Some variability in the uptake was found for each cell line studied. This variability is certainly related to the fluctuations of cultured cells as previously discussed by Lleu and Rebel (1989). However, a notable difference in the uptake level of the three Ishikawa cells grown under identical culture conditions was found. It is reported that fatty acid composition of cellular lipids modulates taurine uptake (Balcar et al., 1980). This is greatly influenced by the culture medium and the serum used. However, this does not apply here since the culture conditions were always identical for the three cell lines. Though we have no explanation for this great difference, which is not related to basal taurine efflux, absent in the three cell lines. Indeed, though statistically significant, the efflux found in Ishikawa-B was not physiologically significant, being too low to account for a real effect on the low taurine uptake in the Ishikawa-B cells.

Though taurine uptake has been studied in other estrogen sensitive tissues such as mammary gland (Shennan and Mc Neillie, 1994; Shennan, 1995; Bryson et al., 2001) or placenta (Iioka et al., 1984; Miyamoto et al., 1988; Karl and Fisher, 1990; Moyer et al., 1992; Ramamoorthy et al., 1993), it is surprising that no study has ever addressed the effect of steroid hormones on taurine uptake. To the best of our knowledge only Lewis et al. (1982) and Lombardini (1986) have shown that administration of estrogen to female rats did not significantly affect taurine concentration either in blood or in urine (Lombardini, 1986). Estradiol affected taurine uptake only in growing Ishikawa-M cells, whereas it notably increased the alkaline phosphatase activity, an estrogen-dependent marker enzyme for endometrial cells, in the three cell lines (Deret et al., in preparation). Moreover, the relations between the estradiol concentration and the level of taurine uptake, and the lack of effect of the anti-estrogen tamoxifen are in favor of a non-genomic action of estradiol on taurine uptake. The uptake of taurine in M cells grown in the presence of 5% NCS or FCS was similar. In contrast to FCS, NCS is physiologically devoid of estrogen. This argues in favor of the non-genomic effect of estradiol. However, estradiol concentration of the FCS batch used in these studies was 600 pg/ml. E2 concentration in the 2 ml medium supplemented with 5% FCS used to culture cells was therefore $0.11\ 10^{-9}$ M, a level too low to affect uptake in Ishikawa-M cells.

As shown for all the cultured cell types studied, taurine uptake in the three Ishikawa cell lines undergoes a regulation process via a phosphorylation pathway. Previously, three kinases, the calmoduline-dependent kinase, PKC and PKA, have been reported for their capacity to modulate taurine uptake (Mollerupt and Lambert, 1996). Our results show that for Ishikawa cells, only PKC was involved in this regulation. As reported for numerous cell types, we also observed that activation of PKC with tetradecanoyl phorbol acetate (TPA) induced a notable decrease in the uptake in the three Ishikawa cell lines. Most of the published data show that TPA inhibits taurine uptake in a large variety of confluent cultures, where most or all the cells are non-growing. In Ishikawa cells, TPA inhibited the uptake only in growing cells and was completely ineffective on resting cultures whatever their density (Table 8). This is not a specificity of Ishikawa cells, since taurine uptake in cultured differentiated non-growing neurons from rats was also not inhibited by TPA (Tschoumkeu-Nzouessa and Rebel, 1996b). Hexadecylphosphocholine, an inhibitor of PKC (Geilen et al., 1991; Uberall et al., 1991) totally inhibited the effect of TPA on taurine uptake. Moreover 4α phorbol didecanoate, a phorbol ester devoid of effect on PKC (Kikkawa et al., 1983) did not affect taurine uptake, thus confirming the implication of PKC in taurine uptake regulation in Ishikawa cells. This regulation was not affected by estradiol, TPA being equally efficient on cells treated or not with E2. Under some circumstances, tamoxifen behaves as a PKC inhibitor (for examples see: O'Brian et al., 1985; Gundimeda et al., 1996; Horgan et al., 1996; Cheng et al., 1998; Williams et al., 2000; Mehrens et al., 2000). However, tamoxifen did not affect the inhibition of taurine uptake induced by TPA under experimental conditions in which this compound acts either as an anti-estrogen or as a PKC inhibitor. This lack of effect of tamoxifen on the PKC triggered modulation of taurine uptake may be due to a change in the lipid environment of PKC, as suggested by Issandou et al. (1990).

The maximal effect of TPA occurred after 150 minutes. This time lapse is in the range of that is usually observed for other cell types. To the best of our knowledge, no study has addressed the reversal of the TPA effect. In Ishikawa cells, reversal of TPA effect rapidly followed

the inhibitory phase. This was also probable for the JAR placenta choriocarcinoma cells (see Table 3 in Kulanthaivel et al., 1991). Reversal of the TPA effect is a relatively fast event compared to other systems such as the reversibility of receptor desensitisation which requires more than 24 hours (see for examples Shifrin and Klein, 1980; Dibner and Insel, 1981). This fast reversal could be a protective mechanism, preventing endometrial cells from being too much depleted of taurine.

If the basal properties of the taurine uptake in the three Ishikawa variants were similar, some notable differences were found in the uptake regulation. These data also complement the results obtained on cell growth modulation showing that although the three Ishikawa cell lines have the same name, in fact they represent three different variants (Deret et al., in preparation). The existence of variants of cell lines with differences in their intrinsic properties is of particular interest. The best documented example concerns the human breast cancer MCF7 cell lines. Various cell lines with the same name, e.g. MCF7, but with different sensitivity towards estrogen and antiestrogens are available (Seibert et al., 1983; Butler, 1986; Osborne et al., 1987; Villalobos et al., 1995; Ram et al., 2000). Nishida et al. (1996) analysing the 25th passage of the Ishikawa line have observed that it was heterogeneous with respect to estrogen sensitivity. Our present study shows that this heterogeneity, which certainly mirrors the *in vivo* tumoral heterogeneity concerns not only the basic properties of the cell lines (e.g. hormone-sensitivity) but also other physiological properties which are involved in the regulation of cellular homeostasis.

References

Adam MF, Dorie MJ, Brown JM (1999) Oxygen tension measurements of tumor growing in mice. Int J Radiat Oncol Biol Phys 45: 171–180

Aquino-Parsons C, Green A, Minchinton AI (2000) Oxygen tension in primary gynaecological tumours: the influence of carbon dioxide concentration. Radiother Oncol 57: 45–51

Balcar VJ, Borg J, Robert J, Mandel P (1980) Uptake of L-glutamate and taurine in neuroblastoma cells with altered fatty acid composition of membrane phospholipids. J Neurochem 34: 1678–1681

Berthois Y, Katzenellenbogen JA, Katzenellebogen BS (1986) Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. Proc Natl Acad Sci USA 83: 2496–2500

Blasig IE, Sporbert A, Utepbergenov DI, Schroeter ML, Mertsch K, Haseloff RP (1998) Cytokine and hypoxia induced lipid peroxidation in astrocytes. Int J Clin Pharmacol Ther 36: 112–113

Borghetti AF, Piedimonte G, Tramagere M, Severini A, Chiringhelli P, Guidotti G (1980) Cell density and aminoacid transport in 3T3, SV3T3 and SV3T3 revertant cells. J Cell Physiol 105: 39–49

Bradley W, Culp L (1974) Stimulation of 2-deoxyglucose uptake in growth-inhibited BALB-C 3T3 and revertant SV40-transformed 3T3 cells. Exp Cell Res 84: 335–350

Brandsch M, Miyamoto Y, Ganapathy V, Leibach F (1993) Regulation of taurine transport in human colon carcinoma cell lines (HT29 and Caco-2) by protein kinase C. Am J Physiol 264: G939–G946

Brown JM, Giaccia AJ (1998) The unique physiology of solid tumors: opportunities and problems for cancer therapy. Cancer Res 58: 1408–1416

Bryson JM, Jackson SC, Wang H, Hurley WL (2001) Cellular uptake of taurine by lactating porcine mammary tissue. Comp Biochem Physiol part B 128: 667–673

Buckler KJ, Vaughan-Jones RD (1994) Effect of hypoxia on membrane potential and intracellular calcium in rat neonatal carotid body type 1 cells. J Physiol 476: 423–428

Bulletti C, Gurpide E, Flamigni C (eds) (1994) The human endometrium. Annals NY Acad Sci 734: 1–500

Butler WB, Berlinski PM, Hillman RM, Kelsey WH, Toenniges MM (1986) Relation of *in vitro* properties to tumoregenicity for a series of sublines of the human breast cancer cell line MCF7. Cancer Res 46: 6339–6348

Cheng AL, Chuang SE, Fine RL, Yeh KH, Liao CM, Lay JD, Chen DS (1998) Inhibition of the membrane translocation and activation of protein kinase C, and potentiation of doxorubicin-induced apoptosis of hepato-carcinoma cells by tamoxifen. Biochem Pharmacol 55: 523–531

Cornfield DN, Stevens T, Mc Murtry IF, Abman SH, Rodman DM (1993) Acute hypoxia increases cytosolic calcium in fetal pulmonary artery smooth muscle cells. Am J Physiol 265: L53–L56

Dibner MD, Insel PA (1981) Growth of C6 glioma cells in serum containing medium decreases β adrenergic receptor number. J Cell Physiol 109: 309–315

Franconi F, Stendardi I, Failli P, Matucci R, Baccaro C, Montorsi L, Bandinelli R, Giotti A (1985) The protective effects of taurine on hypoxia (performed in the absence of glucose) and on reoxygenation (in the presence of glucose) in guinea-pig heart. Biochem Pharmacol 34: 2611–2615

Geilen CC (1991) The phospholipid analogue hexadecylphosphocholine, inhibits protein kinase C *in vitro* and antagonises phorbol ester stimulated cell proliferation. Eur J Cancer 27: 1650–1653

Good NE, Winget GD, Winter W, Connoly TW, Izawa S, Singh RMM (1966) Hydrogen ion buffers for biological research. Biochemistry 5: 467–477

Guillemin K, Krasnow MA (1997) The hypoxic response: huffing and HIFing. Cell 89: 9–12

Gundimeda U, Chen ZH, Gopalakrishna R (1996) Tamoxifen modulates protein kinase C via oxidative stress in estrogen receptor-negative breast cancer cells. J Biol Chem 271: 13504–13514

Han X, Budreau AM, Chesney RW (1996) Role of conserved peptide in taurine transporter inactivation modulated by protein kinase C. J Am Soc Nephrol 7: 2088–2096

Harden TK, Foster SJ, Perkins JP (1979) Differential expression of components of the adenylate cyclase system during growth of astrocytoma cells in culture. J Biol Chem 254: 4416–4422

Hellkamp J, Christ B, Bastian H, Jungermann K (1991) Modulation by oxygen of the glucagon dependent activation of the phosphoenol pyruvate carboxykinase gene in hepatocyte cultures. Eur J Biochem 198: 635–639

Helmlinger G, Yuan F, Dellian M, Jain RK (1997) Interstitiel pH and pO2 gradients in solide tumors *in vivo*: high resolution measurements reveal a lack of correlation. Nat Med 3: 177–182

Herson PS, Lee K, Pinnock RD, Hugues J, Ashford MLJ (1999) Hydrogen peroxide induces intracellular calcium overload by activation of a non-selective cation channel in an insulin-secreting cell line. J Biol Chem 274: 833–841

Hibbard JU, Pridjian G, Whitington PF, Mc Awad AH (1990) Taurine transport in the *in vitro* perfused human placenta. Pediatr Res 27: 80–84

Höckel M, Schlenger K, Knoop C, Vaupel P (1991) Oxygenation of carcinoma of the uterine cervix: evaluation by computerized O_2 tension measurements. Cancer Res 51: 6098–6102

Horgan K, Cooke E, Hallet MB, Mansel RE (1986) Inhibition of protein kinase C mediated signal transduction by tamoxifen. Importance for antitumor activity. Biochem Pharmacol 35: 4463–4465

Hu QH, Wang DX (1994) Both hypoxic endothelial cell conditionned medium and hypoxia elevate intracellular free calcium in pulmonary artery smooth muscle cells. J Tongji Med Univ 14: 200–203

Huxtable RJ (1992) Physiological action of taurine. Physiol Rev 72: 101–163

Iioka H, Moriyama IS, Ichijo M, Inoue M (1984) Placental transport of taurine in brush border microvillous. Sulfur Amino Acids 7: 165–170

Issandou M, Faucher C, Bayard F, Darbon JM (1990) Opposite effects of tamoxifen on *in vitro* protein kinase C activity and endogenous protein phosphorylation in intact MCF7 cells. Cancer Res 50: 5845–5850

Jayanthi LD, Ramamoorthy S, Mahesh VB, Leibach FH, Ganapathy V (1995) Substrate-specific regulation of the taurine transporter in human placental choriocarcinoma cells (JAR). Biochim Biophys Acta 1235: 351–360

Jones DP, Miller LA, Dowling C, Chesney RW (1991) Regulation of taurine transporter activity in LLC-PK1 cells: role of protein synthesis and protein kinase C activation. J Am Soc Nephrol 2: 1021–1029

Karl PI, Fisher SE (1990) Taurine transport by microvillous membrane vesicles and the perfused cotyledon of human placenta. Am J Physiol 258: C443–C451

Kendler BS (1989) Taurine: an overview of its role in preventive medecine. Prevent Med 18: 79–100

Kikkawa U, Takai Y, Tanaka Y, Miyake R, Nishizuka Y (1983) Protein kinase C as a possible receptor protein of tumor promoting phorbol esters. J Biol Chem 258: 11442–11445

Kim HW, Shim MJ, Kim WB, Kim BK (1996) Dexamethasone recovers phorbol-ester induced reduction of taurine transportation in mouse macrophage cell line RAW 264.7. In: Huxtable RJ, Azuma J, Kuriyama K, Nakagawa N, Baba A (eds) Taurine 2 – Basic and clinical aspects. Plenum Press, New York (Adv Exp Med Biol 403: 59–66)

Kulanthaivel P, Cool DR, Ramamoorthy S, Mahesh VB, Leibach FH, Ganapathy V (1991) Transport of taurine and its regulation by protein kinase C in the JAR human placental choriocarcinoma cell line. Biochem J 277: 53–58

Lelong IH, Rebel G (1998a) pH drift of ''physiological buffers'' and culture media used for cell incubation during *in vitro* studies. J Pharmacol Toxicol Methods 39: 203–210

Lelong IH, Rebel G (1998b) CO_2-independant culture media versus ''physiological buffers'' for transport studies performed on cultured cells: discrepancy on taurine uptake measurements. J Pharmacol Toxicol Methods 39: 211–220

Lewis CJ, Shannon B, Kleeman B (1982) Taurine and cystathionine excretion in female rats as influenced by dietary vitamin B6 and estradiol administration. Nutr Rep Int 25: 269–276

Liebowitz SM, Lombardini JB, Allen CI (1989) Sulfone analogues of taurine as modifiers of calcium uptake and protein phosphorylation in rat retine. Biochem Pharmacol 38: 399–406

Lleu PL, Rebel G (1989) Effects of HEPES on the taurine uptake by cultured glial cells. J Neurosci Res 23: 78–86

Lleu PL, Rebel G (1990) Effects of HEPES on the Na^+, Cl^- dependent uptake of taurine and β alanine by cultured glial cells. Modulation by composition and osmolarity of medium. Neuropharmacol 32: 719–725

Loo DDF, Hirsch JR, Sarkar HK, Wright EM (1996) Regulation of the mouse retinal transporter (TAUT) by protein kinases in Xenopus ooytes. FEBS Lett 392: 250–254

Lombardini JB (1986) Taurine levels in blood and urine of vitamine B6-deficient and estrogen treated rats. Biochem Med Metabol Biol 35: 125–131

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Lyng H, Sundfør K, Trope C, Rofstad EK (1996) Oxygen tension and vascular density in human cervix carcinoma. Br J Cancer 74: 1559–1563

Mankovskaya IN, Serebrovskaya TV, Swanson RJ, Valilova GL, Kharlamova ON (2000) Mechanism of taurine antihypoxic and antioxidant action. High Altitude Med Biol 1: 105–110

Mehrens T, Lelleck S, Cetinkaya I, Knollmann M, Hohage H, Gorboulev V, Boknik P, Koepsell H, Schlatter E (2000) The affinity of the organic cation transporter rOCT1 is increased by protein kinase C-dependent phosphorylation. J Am Soc Nephrol 11: 1216–1224

Minyailenko TD, Pozharov VP, Seredenko MM (1990) Severe hypoxia activates lipid peroxidation in the rat brain. Chem Phys Lipids 55: 25–28

Miyamoto Y, Balkovetz DF, Leibach FH, Mahesh VB, Ganapathy V (1988) $Na^+ + Cl^-$-gradient-driven, high affinity, uphill transport of taurine in human placental brush-border membrane vesicles. FEBS Lett 231: 263–267

Mollerup J, Lambert IH (1996) Phosphorylation is involved in the regulation of the taurine influx via the β system in Ehrlich acites tumor cells. J Membrane Biol 150: 73–82

Moyer MS, Insler N, Dumaswala R (1992) The role of chloride in taurine transport accoss the human placental brush-border. Biochim Biophys Acta 1109: 74–80

Nakamura H, Huang SH, Takakura K (1996) High affinity taurine uptake and its regulation by protein kinase C in human glioma cells. In: Huxtable RJ, Azuma J, Kuriyama K, Nakagawa N, Baba B (eds) Taurine 2 – Basic and clinical aspect. Plenum Press, New York (Adv Exp Med Biol 403: 377–384)

Nishida M, Kasahara K, Kaneko M, Iwasaki H (1985) Establishment of a new human endometrial adenocarcinoma cell line: Ishikawa cells containing estrogen and progesterone receptors. Acta Obstet Gynec Jap 37: 1103–1111

Nishida M, Kasahara K, Oki A, Satoh T, Arai Y, Kubo T (1996) Establishment of eighteen clones of Ishikawa cells. Human Cell 9: 109–116

O'Brian CA, Liskamp RM, Solomon DH, Weinstein IB (1985) Inhibition of protein kinase C by tamoxifen. Cancer Res 45: 2462–2465

Ogasawara M, Nakamura T, Koyama I, Nemoto M, Yoshida T (1993) Reactivity of taurine with aldehydes and its physiological role. Chem Pharm Bull 41: 2172–2175

Osborne CK, Hobbs K, Trent JM (1997) Biological differences among MCF7 human breast cancer cell lines from different laboratories. Breast Cancer Res Treat 9: 111–121

Petegnief V, Lleu PL, Gupta RC, Bourguignon JJ, Rebel G (1995) Taurine analog modulation of taurine uptake by two different mechanisms in cultured glial cells. Biochem Pharmacol 49: 399–410

Petronini PG, Piedimonte G, Borghetti AF (1982) The regulation by cell density of aminoacid transport system L in SV40 3T3 cells. Biochem Biophys Acta 693: 13–21

Philibert RA, Dutton GR (1988) Phorbol ester and dibutyryl cyclic AMP reduce content and efflux of taurine in primary cerebellar astrocytes in culture. Neurosci Lett 95: 323–328

Piedimonte G, Borghetti AF, Guidotti GG (1982) Effect of cell density on growth rate and amino acid transport in simian virus 40 transformed 3T3 cells. Cancer Res 42: 4690–4693

Piedimonte G, Baginski I, Silvotti L, Petronini PG, Borghetti AF (1989) Density dependent regulation of amino acid transport in a Burkitt lymphoma cell line. Cancer Lett 47: 121–125

Qian X, Vinnakota S, Edwards C, Sarkar HK (2000) Molecular characterization of taurine transport in bovine aortic endothelial cells. Biochim Biophys Acta 1509: 324–334

Ram PT, Dai YL, Kiefer T, Klotz DM, Spriggs LL, Hill SM (2000) Differential responsiveness of MCF7 human breast cancer cell line stocks to the pineal hormone melatonin. J Pineal Res 28: 210–218

Ramamoorthy S, Kulanthaivel P, Leibach FH, Mahesh VB, Ganapathy V (1993) Solubilization and functional reconstitution of the human placental taurine transporter. Biochim Biophys Acta 1145: 250–256

Rebel G, Haynes L, Lelong IH (1999) Nerve cell culture methodology: the medium environment. In: Haynes L (ed) The neuron in tissue culture. J Wiley & Sons, New York, pp 323–352

Rice-Evans C, Burdon R (1993) Free radical-lipid interaction and their pathological consequences. Prog Lipid Res 32: 71–110

Rodesch F, Simon P, Donner C, Jauniaux E (1992) Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. Obstet Gynecol 80: 283–285

Rose UM, Bindels RJ, Jansen JW, Van Os CH (1993) The effect of L type Ca^{2+} channel blockers on anoxia induced increases in intracellular Ca^{2+} concentration in rabbit proximal tubule cells in primary culture. Pflugers Arch 423: 378–386

Satoh H (1994) Cardioprotective actions of taurine against intracellular and extracellular calcium induced effects. In: Huxtable R, Michalk DV (eds) Taurine in health and disease. Plenum Press, New York, (Adv Exp Med Biol 359: 181–196)

Schaffer S, Soloduskhko V, Kakhiviashvili D (2002) Beneficial effect of taurine depletion on osmotic sodium and calcium loading during chemical hypoxia. Am J Physiol 282: C1113–C1120

Schurr A, Tseng MT, West CA, Rigor BM (1987) Taurine improves the recovery of neuronal function following cerebral hypoxia: an *in vitro* study. Life Sci 40: 2059–2066

Seibert K, Shafie SM, Triche TJ, Whang-Peng JJ, O'Brien SJ, Toney JH, Huff KK, Lippman ME (1983) Clonal variation of MCF7 breast cancer cells *in vitro* and in athymic nude mice. Cancer Res 43: 2223–2239

Semenza GL (1998) Hypoxia inducible factor 1: master regulator of O_2 homeostasis. Curr Opin Genet Dev 8: 588–594

Serebrovskaya TV, Safronova OS, Gordiy SK (1999) Free radical processes under different oxygen supply conditions. Fiziol Zh (Ukr) 45: 92–104

Shennan DB (1995) Identification of a high affinity taurine transporter which is not dependent on chloride. Biosci Rep 15: 231–239

Shennan DB, Mc Neillie SA (1994) High affinity ($Na^+ + Cl^-$) dependent taurine transport by lactating mammary tissue. J Dairy Res 61: 335–343

Shifrin GS, Klein WL (1980) Regulation of muscarinic acetylcholine receptor concentration in cloned neuroblastoma cells. J Neurochem 34: 993–999

Siraki AG, Pourahmad J, Chan TS, Khan S, O'Brien PJ (2002) Endogenous and endobiotic induced reactive oxygen species formation by isolated hepatocytes. Free Radical Biol Med 32: 2–10

Sonnenschein C, Szelei J, Nye TL, Soto AM (1994) Control of cell proliferation of human breast MCF7 cells; serum and estrogen resistant variants. Oncol Res 6: 373–381

Sundfør K, Lyng H, Rofstad EK (1998) Oxygen tension and vascular density in adenocarcinoma and squamous cell carcinoma of the uterine cervix. Acta Oncol 37: 665–670

Tchoumkeu-Nzouessa GC, Rebel G (1996a) Regulation of taurine transport in rat astrocytes by protein kinase C: role of calcium and calmodulin. Am J Physiol 270: C1022–C1028

Tchoumkeu-Nzouessa GC, Rebel, G (1996b) Activation of protein kinase C down regulates glial but not neuronal taurine uptake. Neurosci Lett 206: 61–64

Tseng MT, Lju KN, Radtke WR (1990) Facilitated ERG recovery in taurine-treated bovine eyes, an *ex-vivo study.* Brain Res 509: 153–155

Uberall F, Oberhuber H, Maly K, Zaknun J, Demuth L, Grunicke HH (1991) Hexadecylphosphocholine inhibits inositol phosphate formation and protein kinase C activity. Cancer Res 51: 807–812

Vaupel P, Kallinowski F, Okunieff P (1989) Blood flow, oxygen and nutrient supply, and the metabolic microenvironment of human tumors: a review. Cancer Res 49: 6449–6465

Villalobos M, Brotons JA, Olea-Serrano MF, De Almodovar JMR, Pedraza V (1995) The E screen assay: a comparison of different MCF7 cell stocks. Environ Health Perspect 103: 844–850

Waymouth C (1970) Osmolality of mammalian blood and of media for culture of mammalian cells. *In Vitro* 6: 109–127

Weber MJ, Hale AH, Yau TM, Buckman T, Johnson M, Brady TM, Larossa DD (1976) Transport changes associated with growth control and malignant transformation. J Cell Physiol 89: 711–722

Wersinger C, Rebel G, Lelong-Rebel IH (2000) Detailed study of the different taurine uptake systems in colon LoVo MDR and non MDR cell lines. Amino Acids 19: 667–685

Wersinger C, Rebel G, Lelong-Rebel IH (2001) Characterisation of taurine uptake in human KB MDR and non-MDR tumour cell lines in culture. Anticancer Res 21: 3397–4006

Williams JP, Mc Kenna MA, Thames AM III, Mc Donald JM (2000) Tamoxifen inhibits phorbol ester stimulated osteoclastic bone resorption: an effect mediated by calmodulin. Biochem Cell Biol 78: 715–723

Yedwab GA, Paz G, Homonnai T, David MP, Kraicer PF (1976) The temperature, pH, pressure of oxygen in the cervix and uterus of women and uterus of rats during the cycle. Fertil Steril 27: 304–309

Yoshikawa TY, Furukawa Y, Wakamatsu Y, Takemura S, Tanaka H, Kondo M (1982) Experimental hypoxia and lipid peroxide in rats. Biochem Med 27: 207–213

Zhao P, Huang YL, Cheng JS (1999) Taurine antagonizes calcium overload induced by glutamate or chemical hypoxia in cultured rat hippocampal neurons. Neurosci Letters 268: 25–28

Authors' address: Dr. Gérard Rebel, UPR 9003 CNRS – Institut de Recherche contre les Cancers de l'Appareil Digestif, Hôpital Civil, BP 426, F-67091 Strasbourg Cedex, France,
E-mail: Gerard.Rebel@ircad.u-strasbg.fr