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TAURINE ANALOG MODULATION OF TAURINE UPTAKE BY TWO DIFFERENT MECHANISMS IN CULTURED GLIAL CELLS

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Abstract—Previous data have shown that HEPES, a taurine structural analog, inhibits the uptake of taurine by cultured cells differently, depending on its addition either to the culture medium or to the Krebs–Ringer buffer used for cell incubation during taurine uptake measurements (Lleu and Rebel, *J Neurosci Res* **23**: 78–86, 1989). An extensive study of the effect of numerous other taurine structural analogs on taurine uptake by cultured glial cells was carried out. Our results show that taurine uptake modulation by structural analogs follows two different mechanisms. For the first mechanism, observable after the simultaneous presence of taurine and of its analog during the incubation time of the uptake experiment (10 min), the amine function on the molecule is essential. The sulfonate group could be replaced either by a sulfinic group or by a carboxylic group. β-Alanine, hypotaurine, acetyltaurine, guanidinoethanesulfonate and guanidinopropionate are the most potent inhibitors in this first mechanism. For the second mechanism, which requires the presence of the analog in the culture medium during the 48 hr preceeding the taurine uptake measurement, the simultaneous presence of an amine and of a sulfonate group or of an amine and a sulfinate group is required. Carboxylates are ineffective in modulating taurine uptake in this mechanism. The sulfonate buffers synthesized by Good *et al.* (*Biochemistry* **5**: 467–477, 1966) also affect taurine uptake in both mechanisms.

Key words: taurine analogs; sulfonate buffers; glial cells; transport; uptake

High concentrations of taurine are found in mammalian nervous tissues, particularly in glial cells. Various functions have been attributed to taurine. Beside its general function of cellular osmoregulator, taurine is also a neuromodulator and improves brain development (for review, see Ref. 1). Taurine enters the cell through the very efficient Na^+ Cl^- dependent uptake mechanism [1].

Physiological modulation of this uptake is important for the regulation of some taurine functions such as its osmoregulatory and the neuroregulatory properties. Previous studies have shown that HEPES, a structural derivative of taurine, inhibits taurine uptake in primary cultures of rat glial cells by two different mechanisms: the first is related to the presence of the zwitterion HEPES in the cell culture medium while the second requires its presence in the Krebs–Ringer incubation medium during the taurine uptake measurement [2]. The first has been designated the "slow mechanism" and the second the "fast mechanism" [2]. These terms are not related to any kinetic parameters of taurine uptake, but refer to the compound contact time with cultured cells required for the observation of the modulation phenomenon. Kinetic studies on the effects of HEPES when present in the culture medium indicated that at least 24 hr are required to observe a significant inhibition of taurine uptake [2], whereas the presence of HEPES for 10 min in the incubation medium is sufficient to inhibit taurine uptake by the fast mechanism.

In an attempt to determine if other compounds beside HEPES modulate taurine uptake by these two mechanisms, the effects of numerous compounds have been tested on taurine uptake in the simultaneous (fast mechanism) or sequential (slow mechanism) presence with taurine. In order to differentiate the two mechanisms producing a common effect (i.e. inhibition of taurine uptake) a structure–activity study has been undertaken. We report here, using newly synthesized as well as commercially available compounds, that the structural requirements for taurine analogs to affect taurine uptake are not identical when added either to the glial cell culture medium or the incubation buffer.

MATERIALS AND METHODS

Glial cell cultures. Primary cultures were established by mechanical dispersion from newborn rat hemispheres [3]. Cells were grown in 3.5 cm ø Petri dishes (Costar) in DMEM§ (74–1600 from GIBCO) buffered with 24 mM sodium bicarbonate, supplemented with selected heat inactivated FCS (from J. BOY, Reims, France or GIBCO). The initial 20%

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§ Abbreviations: DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; GABA, γ-aminobutyric acid; GES, guanidinoethanesulfonic acid; GFAP, glial fibrilary acidic protein.

FCS concentration was progressively reduced to 5%. Media were changed every 4 days. The experiments were performed with 15 day old cultures. The cultures used display the classical morphology described by Booher and Sensenbrenner [3]. Under our conditions, cultures reached confluence after approx. 12 days. Morphological aspects as well as reactivity with anti-GFAP showed that more than 95% of the cells are astrocytes. The cultures contained few oligodendrocytes.

Taurine uptake. Taurine uptake was measured as previously detailed [2]. Briefly, the culture medium was removed and the cells were washed twice with a Krebs–Ringer phosphate buffer. The cells were then preincubated in a shaken water-bath at 37° with 3 mL of this buffer for 5 min and after addition of 100 μL of 40 μM cold taurine containing 0.4 μCi [^{3}H]taurine (S.A. = 25.6 Ci/mmole) (final concentration 1.33 μM), cells were incubated for 10 min. After removal of the incubation medium the cells were washed three times with cold saline. They were frozen, treated for 1 hr at 37° with 0.1 N NaOH, then homogenized. Samples were taken for radioactivity and protein determination [4].

Uptake measurements (with taurine analogs). The different compounds to be tested were added, at the required concentration, either to the culture medium 48 hr before taurine uptake determination or to the Krebs–Ringer buffer used to preincubate and incubate cells. The media pH were adjusted to 7.4 with sodium hydroxide. Media osmolarities were checked using a Roebling osmometer.

β-alanine uptake. The β-alanine uptake was measured as described in "Taurine uptake". After a 5 min preincubation in the Krebs–Ringer phosphate buffer, 100 μL of 300 μM cold β-alanine containing 1.2 μCi [^{3}H]-β-alanine (S.A. = 120 Ci/mmole) was added (final concentration 10 μM) to the 3 mL Krebs–Ringer buffer and the cells were incubated for 15 min [5]. After the three cold saline washings, the cells were treated as described in "Taurine uptake".

β-alanine determination in the culture medium. Confluent cultures grown in 10 cm ø Petri dishes were maintained for 2 days in 5 mL/dish DMEM + 5% FCS supplemented or not with 5 mM β-alanine. Culture media were recovered and their β-alanine content determined using a Biotronik 5001 amino acid analyser.

Synthesis of the non-commercially available taurine analogs (see Table 1 for the various structures)

N,N-dimethyltaurine [6], acetyltaurine [7] and GES [8] were prepared following the different procedures described in the literature. The primary (ST 31-84, mp 134°) [9], NH-isopropyl (NCS 703, mp 108°) and piperidino (NCS 713, mp 146°) aminoethanesulfonamides were prepared as hydrochloride salts after removal of the phthaloyl group of their *N*-phthalimido derivatives by means of hydrazine hydrate, as described by Miller *et al.* [9]. The later compounds and other *N*-phthalimido ethanesulfonamides: (MY 117, mp 136°) [10] and NCS 730 (*N*-methylpiperazino, hydrochloride, mp 260°) were prepared following previously described procedures [9–11] starting from β-chloroethylphthalimide [12]. Compounds NCS 703, 713 and 730 were purified by recrystallization from ethanol.

Some of the compounds tested in this study were obtained as Na^+ salts and others as free acids (Table 1). These last compounds acidify the culture or incubation media buffered to pH 7.4 before use.

The statistical significance of differences between two means was evaluated by Student's independent two-tailed *t*-test. A difference in taurine uptake measured in the presence of a tested analog becomes significant when it reaches approx. ±25% of the control value (measurement in the absence of the analog). This significance increases when the percentage of the uptake inhibition or activation increases.

RESULTS

Effect of the ionic species of HEPES

Na^+ activates taurine uptake (for review, cf. Ref. 1). However, the effects of the free acid form of HEPES on taurine uptake were similar after the media were buffered with NaOH or KOH, showing that the addition of a low concentration of sodium ions did not modify uptake (Table 2). Therefore, the media were neutralized with NaOH when required in order to facilitate comparison with the effect of compounds obtained as Na salts.

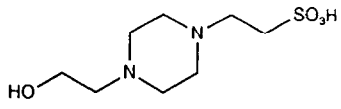
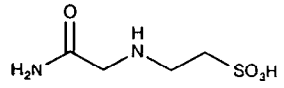
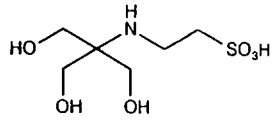
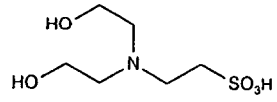
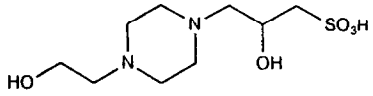
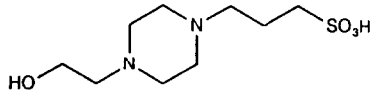
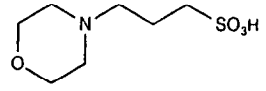
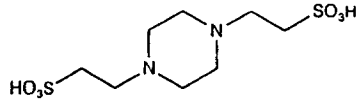
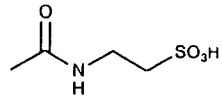
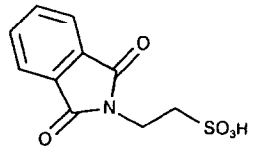
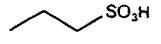
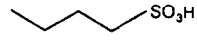
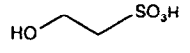
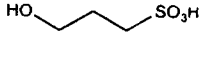
Tables 3 and 4 show the effect of the different compounds when added either to the culture or to the incubation medium by ranking them according to their inhibitory capacity on taurine uptake. Each compound was tested at 1 and 5 mM, minimal and maximal concentrations generally used in this kind of study. The 20 mM concentration was used to compare compounds with the sulfonate buffers.

Various taurine analogs were considered in this study. Some kept their zwitterionic character: e.g. *N*-methyltaurine, *N,N*-dimethyltaurine and GES. Others had their amino group masked in an *N*-acyl moiety, such as acetyltaurine and NCS 731. In some of the tested compounds, the sulfonic acid group was replaced by a non-acidic sulfonamide residue: ST 31-84, NCS 703, NCS 713 and NCS 707. Moreover, taurine isosteres (i.e. isethionic acid, mercaptoethanesulfonate, hypotaurine, aminoethanesulfonate, β-alanine) were also compared with taurine.

Effect of structural analogs added to the culture medium

The first observation is that inhibition was weaker when the analog was present in the culture medium than when it was present in the incubation medium (Tables 3 and 4). Moreover, the inhibitory action of numerous compounds was similar when tested at 1 or 5 (even at 20 mM). Data listed in Table 3 suggest that this system seems to be specific to sulfonic acid derivatives, as the carboxylate isostere (β-alanine) and the phosphonate homologue (aminoethylphosphonate) were found to be inactive. The sulfinic acid derivative hypotaurine was also active. However, it remains possible that it could be partly oxidized to taurine in the culture medium. NCS 713 and NCS 707 were also active, showing that sulfonic acid can

Table 1. Sources and structures of taurine analogs tested in this study

Compound	Origin and form		Structure
HEPES	Free acid	Sigma	
ACES		Sigma	
TES		Sigma	
BES		P.L.B.	
HEPPSO		Sigma	
HEPPS (EPPS)		Sigma	
MOPS		Sigma	
PIPES		Sigma	
N-Acetyltaurine		Synth.	Na^+ 
NCS 731		Synth.	Na^+ 
Propanesulfonic acid		Fluka	Na^+ 
Butanesulfonic acid		Janssen	Na^+ 
Isethionic acid (2-Hydroxyethane sulfonic acid)		Sigma	Na^+ 
3-Hydroxypropane sulfonic acid		EGA Chemie	Na^+ 

Compounds obtained as free acids are indicated: Synth, synthesized in our laboratory; U.S.B., Union State Biochemical Corp. (Cleveland, OH, U.S.A.); Ega Chemie, Steinheim/Albuch (Germany); TCI, Tokyo Kasei Kogyo (Tokyo, Japan); PLB, PL Biochemicals (Milwaukee, WI, U.S.A.).

Table 1. *continued*

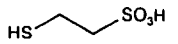
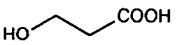
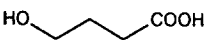
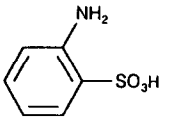
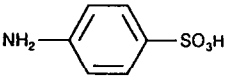
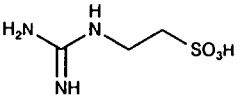
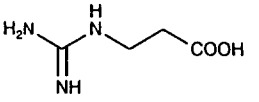
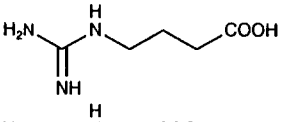
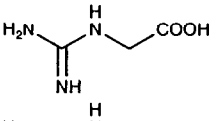
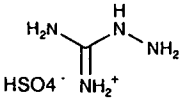
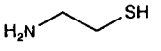
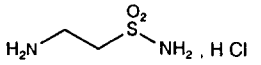
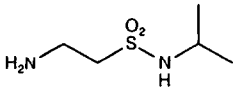
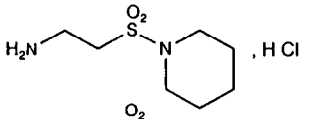
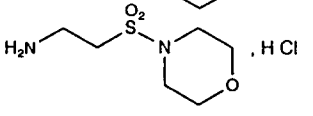
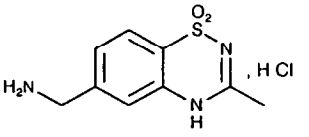
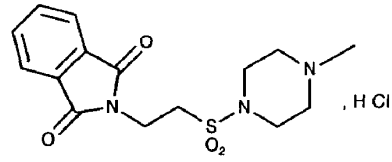
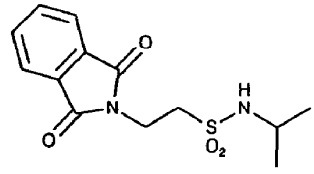
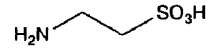
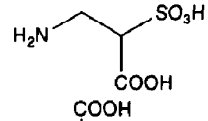
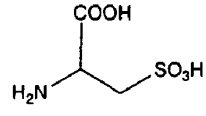
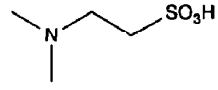
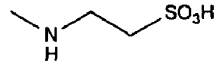
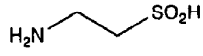
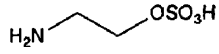
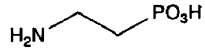
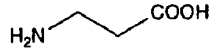
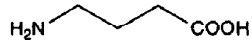
Compound	Origin and form		Structure
2-Mercaptoethane sulfonic acid	Aldrich	Na^+	
3-Hydroxypropionic acid	TCI (Tokyo)		
4-Hydroxybutyric acid	Sigma	Na^+	
2-Aminobenzene sulfonic acid	Aldrich		
4-Aminobenzene sulfonic acid	Sigma		
GES (Guanidinoethane sulfonic acid)	Synth.		
Guanidinopropionic acid	Sigma		
Guanidinobutyric acid	Sigma		
Guanidinoacetic acid	Sigma		
Aminoguanidine sulfate	Sigma		
Thiotaurine	Aldrich	HCl	
ST 31-84	Synth.	HCl	
NCS 703	Synth.	HCl	
NCS 713	Synth.	HCl	
NCS 707	Synth.	HCl	
TAG	M.S.D.	HCl	

Table 1. *continued*

Compound	Origin and form		Structure
NCS 730	Synth.	HCl	
MY 117 (Taltrimide)	Synth.	HCl	
Taurine	Sigma		
α-Sulfo-β-alanine	U.S.B		
Cysteic acid	Sigma		
N-Dimethyltaurine	Synth.		
N-Methyltaurine	Merck		
Hypotaurine	Sigma		
2-Aminoethyl hydrogen sulfate	Aldrich		
2-Aminoethyl phosphonic acid	Aldrich		
β-alanine	Sigma		
GABA	Sigma		
3-Amino-1-propane sulfonic acid	Aldrich		

be replaced by a typical tertiary amide but not by an unsubstituted sulfonamide (compared to its corresponding unsubstituted derivative ST 31-84). The amino function of taurine is also necessary, since its elimination (propane and butanesulfonate) or its replacement by an isosteric unprotonated group such as a mercapto (mercaptoethanesulfonate) or a hydroxyl group (hydroxyethanesulfonate) led to weakly active or inactive compounds. However, the charge of nitrogen in taurine can be incorporated in more (guanidine in GES) or less (acetyl in acetyltaurine) delocalized systems. Moreover, introducing small substituents on the nitrogen of taurine (*N*-monomethyl and dimethyltaurine) produced nearly inactive compounds. The weak inhibitory capacity of α-sulfo-β-alanine was probably

Table 2. Effect of the ionic form of HEPES on the inhibition of taurine uptake by HEPES

Buffer	Neutralization	Taurine uptake (pmol/min/mg protein)	
		Culture medium (A)	Incubation medium (B)
$NaHCO_3$	—	17.8 ± 3.7	17.2 ± 1.0
20 mM HEPES free acid	NaOH 10 N (150 μL)	9.0 ± 0.4†	3.1 ± 0.2*
20 mM HEPES free acid	KOH 10 N (150 μL)	11.8 ± 1.8†	4.7 ± 0.5*

* $P < 0.001$; † $P < 0.05$ compared to control cells cultured in DMEM with no analog.
(A) 13-day-old glial cells were cultured for 2 days in DMEM buffered either with 24 mM $NaHCO_3$ or 20 mM HEPES neutralized to pH 7.4 either with NaOH or KOH. (B) 15-day-old glial cells were incubated in Krebs–Ringer buffer with or without 20 mM HEPES neutralized to pH 7.4 with NaOH or KOH.
Each result is the mean of six values ± SD.

Table 3. Effect of structural analogs added to the culture medium on taurine uptake

Compound	Concentration (mM) in culture medium		
	1	5	20
Taurine	30	19	8
GES	36	22	17
N-Acetyltaurine	42	30	28
Hypotaurine	45	39	16
NCS 713	51	56	21
3-Aminopropanesulfonate	53	46	35
Cysteic acid	—	64	45
NCS 707	—	65	—
2-Aminoethyl hydrogen sulfate	57	69	65
2-Hydroxyethanesulfonate	62	128	120
NCS 730	67	Insoluble	—
NN'-Dimethyltaurine	68	48	45
NCS 703	73	83	36
N-Methyltaurine	74	42	30
Thiotaurine	74	Toxic	—
Aminoguanidine sulfate	75	73	32
α-Sulfo-β-alanine	80	79	53
3-Hydroxypropionate	80	81	89
Guanidinobutyrate	83	71	57
ST 31-84	85	90	126
2-Mercaptoethanesulfonate	85	125	Toxic
2-Aminoethylphosphonate	93	76	68
2-Aminobenzenesulfonate	93	86	80
Guanidinoacetate	94	120	132
NCS 731	99	65	16
MY 117	99	Insoluble	—
4-Aminobenzenesulfonate	104	—	85
β-Alanine	128	131	67
TAG	138	85*	—
Guanidinopropionate	156	137	131
GABA	—	119	105
3-Hydroxypropanesulfonate	—	118	90
4-Hydroxybutyrate	—	—	112
Propanesulfonate	—	—	133
Butanesulfonate	—	—	143

* Tested at 2 mM, insoluble at 5 mM. Statistical significance of differences from the corresponding control: $P < 0.05$ at least for compounds inhibiting or increasing taurine uptake by more than 25%.
Compounds are ranked according to their inhibitory capacity obtained with 1 mM, the concentration classically used.
Results are expressed as % of control uptake.
Each result is the mean of at least four values which did not differ by more than 10%.

Table 4. Effect of taurine analogs added to the incubation medium on taurine uptake

Compound	Concentration (mM) in incubation medium		
	1	5	20
Hypotaurine	2	—	4
Taurine	3	1	0.5
β-Alanine	6	—	—
GES	14	2	—
N-Acetyltaurine	14	6	3
Guanidinopropionate	17	12	2
Guanidinobutyrate	32	10	7
GABA	34	12	5
N-Methyltaurine	36	13	5
3-Aminopropanesulfonate	47	20	7
NCS 713	51	42	8
NN'-Dimethyltaurine	53	35	13
ST 31-84	55	31	16
NCS 703	56	35	41
Guanidinoacetate	58	28	16
TAG	61	45*	—
2-Aminoethyl hydrogen sulfate	64	33	15
α-Sulfo-β-alanine	64	47	3
Cysteic acid	75	88	98
2-Mercaptoethanesulfonate	82	—	99
NCS 730	84	Insoluble	—
MY 117	86	Insoluble	—
Thiotaurine	89	104	65
NCS 731	90	48	74
2-Aminobenzene sulfonate	91	78	77
2-Hydroxyethanesulfonate	93	109	87
3-Hydroxypropionate	98	101	71
2-Aminoethylphosphonate	101	51	50
Aminoguanidine sulfate	102	78	36
NCS 707	—	60	—
4-Aminobenzene sulfonate	—	102	82
3-Hydroxypropanesulfonate	—	103	71
Propanesulfonate	—	—	92
Butanesulfonate	—	—	94
4-Hydroxybutyrate	—	—	112

* Tested at 2 mM, insoluble at 5 mM. Statistical significance of differences from the corresponding control: $P < 0.05$ at least for compounds inhibiting or increasing taurine uptake by more than 25%.

See legend of Table 3.

Results are expressed as % of control uptake.

Each result is the mean of at least four values which did not differ by more than 10%.

related to the antagonist effect of the sulfonate and carboxylate moieties. Finally, the inability of the two aminobenzenesulfonates to modify taurine uptake shows that steric effects and geometrical considerations may be crucial for action.

The absence of effect of β-alanine added to the culture medium on taurine uptake could be related either to a highly efficient uptake of this amino acid by glial cells or to its catabolism in the culture medium. Confluent primary glial cultures were maintained for 2 days in DMEM + 5% FCS with or without 5 mM β-alanine. Most of the added β-alanine (4.7 ± 0.2 mM) was recovered in the culture medium after 2 days. This recovery shows that the β-alanine concentration present in the culture medium after 2 days was sufficient to completely inhibit taurine influx, if the slow and the fast mechanisms were similar. The absence of effect of β-alanine (or other carboxylated taurine analogs) supplemented to the culture medium could not be related to their withdrawal during the washing procedure which precedes taurine uptake measurement, as all inhibitory sulfonate taurine analogs (such as GES) remain inhibitors under the same conditions.

Effect of compounds present in the incubation medium

Comparison of data listed in Tables 3 and 4 suggested that the fast system is less specific as the carboxylic isostere β-alanine was highly potent. Higher homologues such as GABA continued to be active. An acidic group in the β or in γ position to an amino group seems adequate for recognition by the "taurine transporter recognizing site". Some of the compounds that negatively affected taurine uptake when added to the culture medium behaved similarly when present in the incubation medium. None of the compounds tested in the incubation medium increased uptake significantly.

Table 5. Effect of sulfonate buffers on taurine uptake

Compound (mM)	Culture medium 20 (with $NaHCO_3$)	20 (alone)	Incubation medium 20
ACES	73	68	49
HEPPSO	46	51	56
BES	80	72	64
HEPPS	38	41	73
MOPS	—	77	81
TES	138	—	91
PIPES	73	99	89

When tested on the slow mechanism, 13-day-old glial cells were cultured for 2 days either in DMEM buffered with 20 mM of the sulfonate buffer or DMEM buffered with 24 mM bicarbonate + 20 mM of the sulfonate buffer.

Results are expressed as % of control uptake. Each result is the mean of four values.

Table 6. Comparative effect of some compounds on the uptake of β-alanine and taurine

Compounds	Culture medium β-Alanine	Taurine	Incubation medium β-Alanine	Taurine
GES	22	22	6	2
N-Acetyltaurine	27	30	11	6
Hypotaurine	59	45	15	2
3-Aminopropanesulfonate	50	46	21	20
Taurine	64	30	20	3
Guanidinopropionate	94	137	7	12
GABA	—	119	14	12
α-Sulfo-β-Alanine	103	79	55	47
Aminoguanidine sulfate	79	73	64	78
ST 31-84	103	90	64	31
β-Alanine	—	128	12	6
ACES	59	73	61	49

Results are expressed as % of control uptake. Each result is the mean of four values. Hypotaurine, β-alanine and taurine were tested at 1 mM and ACES at 20 mM. Other compounds were used at 5 mM concentration.

Effect of sulfonate buffers

Sulfonate buffers can be tested in the absence of bicarbonate in the culture medium only at a 20 mM concentration (Table 5), their buffering capacity being inefficient at lower concentrations. However, even at this concentration, the buffering capacity of these compounds was very weak and the pH of the culture medium was below 7.0 after 48 hr in culture. Lleu and Rebel [5] have previously shown that the pH decrease in the culture medium when HEPES was used is not the cause of the observed taurine uptake inhibition. Comparison of the effect of HEPES added to unbuffered culture medium or to a medium buffered with 2 g/L sodium bicarbonate showed that the inhibitory effect of the sulfonate compound was lower in the latter case [5]. Nevertheless, for the other tested sulfonate buffers, the presence or absence of bicarbonate in the culture medium did not modify the inhibitory effect. The inhibitory potency of the buffer decreased notably when the piperazine ring was replaced by an alkyl chain.

Comparative effect of some compounds on the uptake of β-alanine and taurine

Table 6 shows that the different compounds tested inhibited the uptake of taurine and β-alanine by glial cells in a similar fashion. This is at variance with the results obtained with HEPES, which affects β-alanine uptake only when present in the culture medium [5].

Effect of HEPES and GES on primary and secondary cultures

Figure 1 shows that the inhibitory effect of HEPES disappeared after a culture replication, while that of GES remained quite similar in the two types of culture.

DISCUSSION

In this study, we attempted to determine the structural requirements for compounds to affect taurine uptake by a "fast mechanism" (incubation conditions) or a "slow mechanism" (presence for

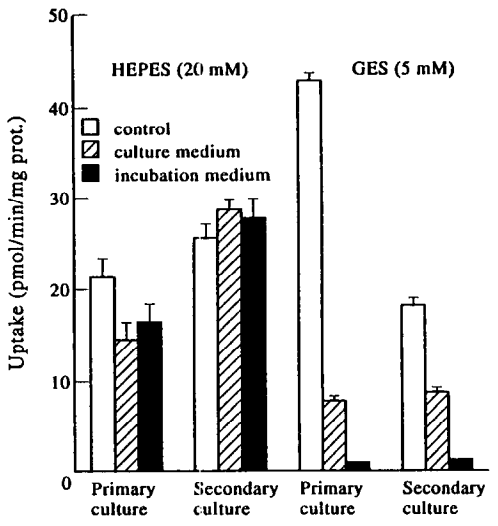


Fig. 1. Effect of HEPES and GES on taurine uptake by primary and secondary glial cell cultures. 13-day-old rat glial cells were changed for 2 days with:—DMEM, supplemented with 5% FCS, buffered with 24 mM $NaHCO_3$ (control HEPES or GES) then incubated with Krebs–Ringer buffer,—DMEM, supplemented with 5% FCS, buffered with 24 mM $NaHCO_3$ + 5 mM GES then incubated with Krebs–Ringer buffer (GES medium),—DMEM, supplemented with 5% FCS, buffered with 24 mM $NaHCO_3$ and incubated with Krebs–Ringer buffer + 5 mM GES (GES incubation medium),—DMEM, supplemented with 5% FCS, buffered with 24 mM $NaHCO_3$ and incubated with Krebs–Ringer buffer + 20 mM HEPES (HEPES incubation medium),—DMEM, supplemented with 5% FCS, buffered with 20 mM HEPES and incubated with Krebs–Ringer buffer (HEPES medium). Secondary cultures: a part of the control cultures (whose culture medium was buffered with sodium bicarbonate) were dissociated by trypsin–EDTA on day 15. Cells were replicated at low density and grown to confluence with DMEM-bicarbonate + 5% FCS. Cells were treated as the primary cultures for 48 hr with GES or HEPES. Results represent two different experimental sets of four values ± SEM.

48 hr in the culture medium). We also tried to provide evidence that these two mechanisms are distinct.

Concerning the fast mechanism, taurine decreased dramatically its own uptake. Cultured glial cells can accumulate high amounts of taurine, more than 20 mM [13], indicating that the observed inhibition was only linked to an isotopic dilution and not to another phenomenon.

It is generally agreed that taurine, β-alanine and hypotaurine enter cells via the same transporter [14–16]. This was established by comparing the concentration of each compound necessary to inhibit the uptake of one of the two other amino acids. The uptake of taurine and β-alanine was similarly inhibited by the different compounds we tested, the only exception being ST 31-84. This shows that although the fast and slow mechanisms were different, both affected the β-amino acid carrier system.

Hypotaurine and β-alanine have been largely used to determine the characteristics of taurine uptake. Our results agree with those obtained by others on different cell types [15–22] with the exception of isolated liver cells [23] and a kidney cell line [24], where these two amino acids behaved as mild taurine uptake inhibitors.

Like β-alanine, GABA has also been thought to be taken up by the taurine uptake system [25]. When tested at a concentration of approx. 1 mM, taurine uptake inhibition ranged from 30 to 70% depending on the cells tested [15–22, 26, 27]. Furthermore, GABA has been shown to slightly increase the uptake of taurine in mouse glial cells [28] and rat glioma cell lines [29]. Our results on rat glial cells were close to those found by Holopainen and Kontro [22] on the same cultures. Under our conditions, the inhibition obtained with aminopropanesulfonic acid, a structural analog of both taurine and GABA, was similar to that reported by Holopainen and Kontro [22], Borg *et al.* [17] on nervous cells and Lambert [21] on Ehrlich ascitic cells.

Besides GABA, two other taurine analogs in which the acidic function was replaced either by a phosphonic acid or a thiol were previously studied. In agreement with our results, 1 mM aminoethylphosphonic acid has practically no effect on taurine uptake by Ehrlich cells [21], rabbit ciliary cells [27], rat glioma cells [25], the only uptake system slightly sensitive to this drug being that of rabbit lens [20].

Different *N*-substituted taurine molecules were tested. The simplest one, *N*-methyltaurine, behaved, under our conditions, as a mild uptake inhibitor. Similar results were previously obtained on other cell types [16, 20, 27]. GES is the most widely used *N*-substituted taurine analog. Though considered as a powerful taurine uptake inhibitor [12], its effects on cell culture have been only scarcely tested. In agreement with the results of Holopainen and Kontro [22], we found that GES was as potent as β-alanine or hypotaurine on the taurine uptake by glial cells. GES also strongly inhibits taurine uptake in lymphoblastoïd cells [26], though to a lesser extent than these two compounds. In contrast, GES only behaves as a mild inhibitor of taurine uptake by liver cells [23]. No compound in which the sulfonate group is replaced by a sulfonamide, substituted or not, has been previously tested on cultured cells. MY 117, a *N*-substituted ethylsulfonamide derivative was shown not to significantly affect the taurine uptake in mouse cerebral slices [30]. This result does not notably differ from the one we obtained with glial cells.

Some of the compounds discussed above as well as other taurine analogs such as acetyltaurine or aminoethyl hydrogen sulfate have been studied by Hruska *et al.* [31]. The low concentration used by these authors (0.1 mM) could explain their inability to modify taurine uptake.

Among the different compounds tested, TAG is known to antagonize *in vivo* some of the physiological effects of taurine [32–35]. Furthermore, MY 117 is known for its anticonvulsant action [30]. At the concentrations which can be used, these compounds were ineffective via the slow mechanism and only

mildly affected, if at all, uptake via the fast mechanism. As discussed above, all the results we obtained on cultured glial cells are in general agreement with the effect of the same analogs when tested on other tissues or cells. This shows that taurine uptake will probably be similarly modulated by the analogs in most vertebrate cell types. Results obtained with TAG would therefore indicate that this compound interferes *in vivo* with taurine action by a mechanism unrelated to the uptake of the amino acid.

Furthermore, it is also possible that some compounds, such as MY 117, could act on other receptors or transporters (such as GABA or benzodiazepines). Moreover, the putative existence of another transport system which recognizes sulfonate derivatives could be hypothesized according to studies on the uptake of propionylcarnitine taurinamide (ST 520. Sigma Tau) by cultured brain cells [36] and of taurine chloramine by macrophage cell lines [37].

The comparison of the inhibitory potency of compounds such as β-alanine, guanidinopropionic acid, guanidinobutyric acid or γ-aminobutyric acid, added either to the culture or to the incubation medium, confirms that the slow and fast taurine uptake inhibitory mechanisms previously described are distinct. The experiment with β-alanine demonstrates that its ineffectiveness is not the result of its disappearance from the culture medium, as almost all β-alanine added to the culture medium is still present after 48 hr. This result confirms our previous observations that β-alanine is not catabolized in the culture medium [38]. Moreover, β-alanine and taurine are taken up by the same system [14–16]. This has also been demonstrated for GES and taurine [8]. Recent observations have shown that β-alanine and GES similarly inhibit the cloned taurine transporter [39–40], confirming that β-alanine and GES are probably taken up by cells using the same β-amino acid transporter. We have not presently been able to prove that the respective association of β-alanine and GES to the transporter are different. Under the above described experimental conditions, β-alanine, but not GES, might have been dissociated from the recognition site of the transporter during the washing steps preceeding incubation in the Krebs–Ringer buffer.

Lleu and Rebel [5] concluded that HEPES behaved differently when tested either on taurine or β-alanine uptake. However, the comparison of the inhibitory effect of HEPES and GES on taurine uptake by primary and secondary glial cell cultures leads us to think that some sulfonate buffers probably affect taurine uptake differently than the other taurine analogs.

In conclusion, a notable modification either of the sulfonate group (amidation), the amino group (inclusion of the nitrogen into a ring) or the aliphatic moiety of the taurine molecule gave only moderately or poorly active compounds. Under our experimental conditions, the structural requirements for a compound to affect taurine uptake by the "fast" mechanism are:

—a sulfonic, sulfinic or carboxylic group,

—a primary amine, *N*-acetyl or guanidine group separated by two non-substituted carbon atoms.

The structural requirement to modulate taurine uptake via the "slow" mechanism is more stringent for the acidic function, because only a sulfonic or a sulfinic group is tolerated, the other structural characteristics (basic group and aliphatic moiety) being similar to those allowing inhibition by the fast mechanism.

Our results extend the previous observations [41] and show that taurine uptake by cultured rat glial cells could be modulated by at least three different mechanisms:

—a direct action on the taurine transporter recognition site (β-alanine, GES, acetyltaurine: fast effect),

—an action which probably requires one or more intermediates, modulating the efficiency of the taurine transporter to take it up (all the compounds acting by the slow mechanism),

—some sulfonate buffers, like HEPES, could modulate taurine uptake through a third mechanism which is still unclear.

Numerous trials attempting to reduce taurine concentration in tissues have been carried out. Treatment of animals or cultures with GES decreases taurine uptake efficiently [1, 42]. Surprisingly, administration of β-alanine is less [43] or not at all efficient [44]. These observations could indicate that *in vivo*, a compound modulates taurine uptake using the slow but not the fast mechanism. This would explain why GES is effective and β-alanine is not.

In this paper we have only studied the effects of different taurine analogs on its uptake by cultured glial cells. Preliminary results have also shown that inhibitors of taurine uptake by the slow mechanism also decrease the taurine content of the glial cells in agreement with the previously published data [1, 42]. One of the ubiquitous roles of taurine in mammalian cells is to regulate cellular osmolarity [45, 46]. It bears investigating some of the potent inhibitors of uptake such as GES, which enters mammalian cells [1, 46], could be implicated in such regulation rather than taurine.

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