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Evaluation of the osmoregulatory function of taurine in brain cells

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Summary. Homogenous primary cultures of mouse astrocytes and cortical neurons were used to clarify the role of taurine in ion and osmoregulation in the CNS. This study indicates that both neurons and glial cells have uptake systems for taurine. The cell water content does not change during loading of cells with taurine. Chemical analysis indicates that part of the accumulated taurine is metabolized and that the product(s) are stored in the cells. Extracellular taurine (1 mM) has no effect on K^+, Na^+, Cl^-, or Ca^{2+} movements in astrocytes. However, astrocytes loaded to a taurine content which corresponds a concentration of 60 mM (corresponds to normal mouse cortex levels) show a 50% reduction in their K^+ accumulation by carriers and a 100% increase in Ca^{2+} turnover rates. Movements of Ca^{2+} and K^+ are involved in neurotransmission. It appears that taurine stored in glial cells, has an important effect on ion homeostasis in the CNS and may act indirectly on neuronal excitability.

Key words: Astrocytes – Calcium ion – Homeostasis – Taurine – Volume control

Introduction

The role of taurine (2-aminoethanesulfonic acid) in cellular interactions in the central nervous system is not yet clear. It has important effects on the excitability of neurons, however, it fails to meet essential criteria to be considered as an amino acid transmitter. In general taurine decreases neuronal discharges (Oja and Kontro 1983) and increases neuronal Cl^- permeability (Okamoto et al. 1983a) and acts as an anticonvulsant (Van Gelder 1978). High-affinity, sodium-dependent taurine uptake systems have been demonstrated for synaptosomes, brain slices, cultured neurons and astrocytes (Schousboe et al. 1976; Martin and Shain 1979; Borg et al. 1979; Kontro and Oja 1983; Holopainen 1984; Holopainen and Kontro 1986). However, one crucial criterion for a transmitter substance has not been satisfied: A calcium-dependent release in a neuronal pathway where taurine has a transmitter-like action has not been demonstrated to date. For this reason a more general neuromodulator role is postulated for taurine. Taurine can be accumulated to tissue concentrations as high as 50 mM (Voaden et al. 1977; Huxtable 1982) and it is released from glial cells upon activation of their β-adrenergic receptors (Shain et al. 1986). Taurine has been proposed as a membrane stabilizer (Lopez-Colome 1982) and a general regulator of ionic fluxes (Pasantes-Morales 1982). Recently, a role as an osmotic effector (Thurston et al. 1980; Van Gelder 1983; Van Gelder and Barbeau 1985) was proposed. In this study we tried to investigate the actions of taurine as an osmo- and ionregulator using primary cultures of neurons and of astrocytes. We first investigated the capacity of these cells to store taurine and the effect of this stored taurine on cellular water content. Glial cells are considered to be the main site of ion homeostasis in the CNS, therefore, we examined the effect of taurine on potassium, sodium, chloride and calcium, fluxes of astrocytes. The effects of extracellular and intracellular stored taurine were studied separately.

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Material and methods

Cell cultures

Cortical neurons and astrocytes in primary cultures from Swiss mice were used. The cultures consisted of monotypic monolayers and had a purity of at least 95% (astrocytes) and 85% (neurons). Their preparation and characterization is described in Hertz et al. (1985).

General protocol for transport studies

The studies were done with the monolayer cultures attached to their culture dishes. For all experiments the growth medium was replaced by bicarbonate buffered salt solution (bubbled with a mixture of 95%/5% air/CO_2). The composition of the salt solution was (in mM): NaCl 123, $NaHCO_3$ 26, KCl 5.4, NaH_2PO_4 1, $MgSO_4$ 0.8, $CaCl_2$ 1.8, glucose 10. If an incubation took longer than two min culture dishes were placed in a 5% CO_2-incubator (37° C). Otherwise they were on the surface of the waterbath (37° C). In many cases the cultures were preincubated in salt solutions before adding solution containing a radiotracer. The exposure time to this salt solution containing radiotracer (= uptake time) varied according to experimental conditions between 10 s and 5 h (see below). The uptake period was usually terminated by washing with an ice cold (1° C) sucrose solution (300 mM). Each wash took roughly 4 s. The cells were then scraped in 1 ml 1N NaOH and divided for protein (Lowry) and radioactivity determination. The results were expressed as nmol substance (calculated from the concentration of the radioactivity in the uptake salt solution) per mg cell protein. The specific protocols for each of the radiotracers are described below.

Taurine

^{3}H-taurine (specific radioactivity 0.17–1.7 μC/ml) and non-radioactive taurine were combined to give the desired final concentration in the salt solution. Taurine uptake was terminated with seven washes in the salt solution (37° C). The samples were counted for radioactivity in a Beckman LS 9800 liquid scintillation counter with correction for quenching.

Potassium

Two different experimental settings for measuring potassium transport in cultured astrocytes were used.

a) Unidirectional K^+ influx through K^+ channels. During steady-state (with respect to ion distribution) small amounts of radioactive ^{42}K were added to the external solution and the accumulation of radiotracers in the cells was measured. The turnover of K^+ at steady-state is very high: the rate constant amounts to 2.7 min^{-1}, which means that the total K^+ content exchange with the external medium about three times per minute. This reflects the very high and exclusive K^+ permeability, which is a general feature of mammalian glial cells (Coles 1986). The unidirectional influx was measured for 10 s. This is the rectilinear part of the ^{42}K accumulation when it is not yet affected by efflux and therefore the measured accumulation of ^{42}K reflects inward transmembrane movements. In mouse astrocytes 95% of this unidirectional influx is sensitive to 50 μM barium, about 3% to ouabain and 2% furosemide (Walz 1986). This means most of the K^+ transfer in steady state is through barium-sensitive channels and can be measured by exposure to ^{42}K for 10 s.

b) Net accumulation of K^+ through carriers. In these experiments, the cells were in equilibrium (with respect to their ion composition) at 3 mM external K^+. ^{42}K was added at least 10 min before the experiment to allow equilibration. The external [K^+] was increased to 12 mM (the physiological ceiling level in the brain) by adding KCl and ^{42}K, so that the specific radioactivity μCi ^{42}K/mol ^{39}K remained constant. This method estimates changes in K^+ content rather than unidirectional K^+ fluxes. The radiotracer was present in 1–10 μCi/ml salt solution. Uptake was terminated by three washes in sucrose solution (1° C). The radioactivity was measured in a Packard Model 5650 Auto Gamma Counter.

Sodium

^{22}Na was present in a specific radioactivity of 1.2 μC/ml. The sodium turnover is rapid, although not as fast as K^+ (see Walz and Hertz 1984). However, because the Na^+ content of the astrocytes is low (one sixth of K^+) the rectilinear part of the ^{22}Na accumulation is 10 s. After 10 s exposure the extracellular Na^+ was removed by seven washes in sucrose solution (1° C). Radioactivity was measured as for ^{42}K.

Chloride

^{36}Cl was present at a specific activity of 0.5 μCi/ml. The rectilinear part of Cl^- uptake is 2 min. The cells were washed seven times with sucrose solution (1° C) to remove extracellular Cl^- (Walz and Hertz 1983a). Radioactivity was measured as for ^{3}H-taurine (using a ^{36}Cl program).

Calcium

^{45}Ca was used to trace calcium movements at a specific activity of 1 μCi/ml. Calcium entry through its channels is relatively fast. The time course of calcium uptake has several rate constants. The fastest one (1.4 min^{-1}) corresponds to Ca^{++} entry through channels, the rectilinear part of ^{45}Ca accumulation is 10 s. For determination of total cellular calcium a 5 h Ca^{++} incubation was selected to load these cells so that several intracellular storage compartments would be included (Walz and Wilson 1986). The ^{45}Ca incubation was stopped by removing Ca^{2+} containing salt solution with five washes of sucrose solution (1° C). Lack of Na^+ in the washing fluid prevented efflux of Ca^{++} by Na^+/Ca^{++} exchange during washing procedure and inhibited ATP dependent extrusion of Ca^{++} (due to low temperature). The sixth wash with sucrose containing 2 mM EGTA was used to remove extracellular bound Ca^{++}. Measurement of radioactivity was with the same procedure as for ^{3}H-taurine (using a ^{45}Ca Program).

Intracellular water space

^{14}C-urea penetrates cell membranes and is not metabolized (Martin and Shain 1979). Extracellular urea equilibrates within two min and reaches the same concentration in the cellular water and the extracellular salt solution. Cells were exposed for 5 min to ^{14}C-urea (specific activity: 1.5 μCi/ml) and then were washed three times with sucrose solution (1° C). The remaining urea was counted as ^{3}H-taurine using a ^{14}C program. Urea removal from the monolayer culture dish has two rate constants, a fast and a slow one. The fast one corresponds to extracellular urea washout and the slow one to efflux of intercellular urea during washing. The urea content of the cells prior to washing was calculated using the slow time constant and the total urea in the cells after three washes (Walz 1987). The water content of the cells was calculated from the radioactivity of ^{14}C-urea in a known volume sample of the incubation salt solution (assuming urea in intracellular water has the same concentration as urea in the salt solution). The intracellular water space was represented as μl cellular water per mg cell protein.

Chemical measurements of cellular taurine content

Taurine analysis was carried out using an HPLC procedure as described by Geddes and Wood (1984). Two model 6000 A pumps, a model 660 solvent programmer, a U6K injector, and a

Table 1. Uptake velocities of cultured neurons and astrocytes for taurine. The values are in nmol taurine × mg^{-1} protein × min^{-1} and are calculated from a 10 min uptake period (n = 3, ± SEM)

Taurine concentration	Neurons	Astrocytes
10 μM	2.3 ± 1.0	0.9 ± 0.4
100 μM	2.7 ± 0.4	1.1 ± 0.1
1 mM	5.5 ± 0.5	1.7 ± 0.5
10 mM	15.2 ± 2.8	7.0 ± 0.7
50 mM		12.9 ± 0.2

μ Bondapak C18 column (Waters Associates) were used. The column effluent was monitored with a Schoeffel Instruments model FS970 spectrofluoro monitor fitted with a 5 μl flow cell. The excitation wavelength was 340 nm and the emission wavelength was 418 nm. A Spectra-Physics SP 4100 computing integrator was connected to the detector. The mobile phase consisted of methanol-potassium acetate (pH 5.55; 0.1 M) and was run at 1.5 ml/min in a linear step (15 min) from 25% to 70% methanol followed by an isocratic hold for a further 5 min. Derivatization of the amino acids was carried out as described by Geddes and Wood (1984).

Materials

^{3}H-taurine, ^{14}C-urea, ^{42}K (as KCl) and ^{45}Ca (as $CaCl_2$) were purchased from DuPont Canada (NEN Products), Lachine, P.Q. ^{22}Na (as NaCl) and ^{36}Cl (as NaCl) were purchased from Amersham Canada, Oakville, Ontario. Nonradioactive taurine and EGTA were from Sigma Chemicals, St. Louis.

Results

This first series of experiments was done to establish some groundwork and determine the time parameters and taurine concentrations which are relevant for the experiments on ionic effects of taurine. The capacities of glial cells and neurons for taurine accumulation and storage were investigated by examining the velocity of taurine uptake and the amount of taurine stored in the cells during exposure to extracellular taurine. This was investigated by using radioactive taurine (exogenous determination) and HPLC analysis of intracellular taurine (endogenous determination). The effects of extracellular taurine concentrations on cell volumes were also investigated. Secondly, experiments were done to investigate the fate of taurine taken up by the cell. Is it free and contributing to cell osmolarity, is it sequestered or is is metabolized?

Taurine transport and metabolism

Uptake velocities. Astrocytes and neurons were exposed for various periods to different concentrations of extracellular taurine. For both cell types, it was found that a 10 min uptake period was well within the rectilinear part of taurine uptake into the cells (taurine concentrations ranging from 10 μM to 10 mM). Table 1 gives an overview of the calculated velocities for taurine uptake.

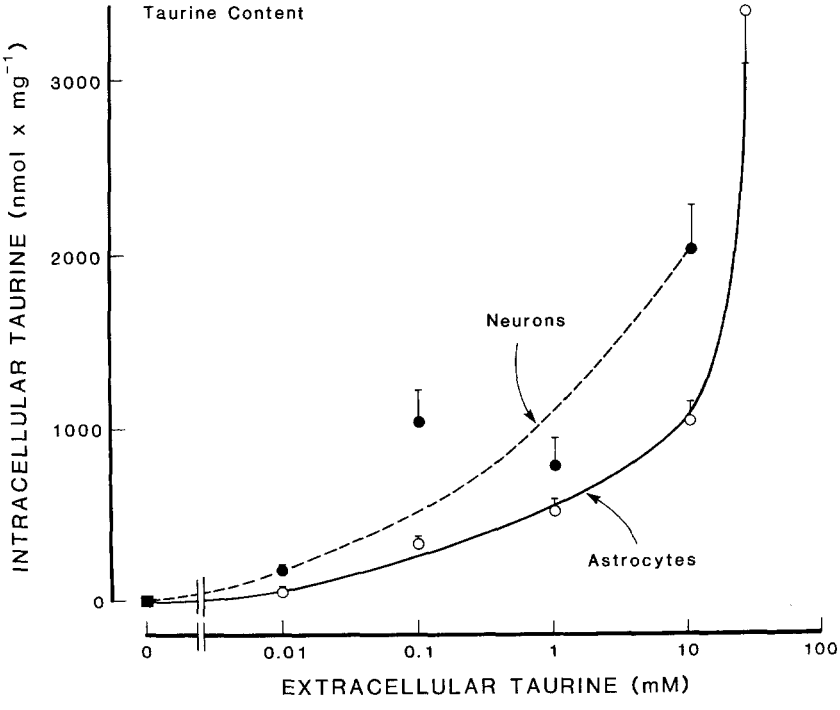

Fig. 1. Taurine content of neurons and astrocytes after 5 h of exposure to different extracellular concentrations of taurine (n = 5; S.E.M. indicated)

Table 2. Comparison of exogenous and endogenous measured taurine in astrocytes and neurons after a 5 h period in salt solution with or without 1 mM taurine (n = 3)

	Astrocytes	Neurons
No external taurine		
exogenous	0	0
endogenous (nmol × mg^{-1})	116.6 ± 7.9	77.3 ± 8.3
concentration (mM)	23.3	9.7
1 mM external taurine		
exogenous (nmol × mg^{-1})	521.8 ± 69.9	772.6 ± 148.2
endogenous (nmol × mg^{-1})	299.3 ± 7.0	146.4 ± 19.2
concentration (mM)	60.0	18.0

Taurine content. The taurine content after 5 h of exposure to various external taurine concentrations was measured in two different ways. First, the cells were exposed to various concentrations (10 μM–50 mM) of radiolabelled taurine. The accumulation of the radioactivity was measured and the results are shown in Fig. 1. Secondly, the cells were exposed to 1 mM non-labelled taurine and the amount of accumulated taurine was measured in the cells chemically. We found first that cultures in growth medium have an endogenous taurine content of 128.6 ± 5.3 nmol × mg^{-1} (astrocytes) and 66.0 ± 11.1 nmol × mg^{-1} (neurons). This corresponds intracellular taurine concentrations (see below) of 25.7 mM (astrocytes) and 8.3 mM (neurons). If taurine was present during this time at a concentration of 1 mM, the amount of chemically detectable taurine in the cells was lower than the content calculated from the accumulated radioactivity. The results are compared in Table 2.

Volume and taurine accumulation. Neurons and astrocytes were loaded for 5 h to different intracellular levels by exposure to different concentrations of extracellular taurine. The cellular volume was measured to see if it changes with different amounts of taurine loading. The results are presented in Fig. 2. There is a small but significant increase of the neuronal volume of about 30% in the presence of extracellular taurine, which remained constant throughout the range (10 μM to 10 mM) tested. The volume of astrocytes remained virtually unchanged during taurine loading as compared with controls.

Taurine concentration. The taurine concentration in neurons and astrocytes was calculated from the endogenous measured taurine contents (Table 2) and corresponding cell water contents (Fig. 2) of the cultures using the following formula:

$$\text{apparent taurine concentration (nmol} \times \mu l^{-1}) = \frac{\text{taurine content (nmol} \times mg^{-1})}{\text{cellular water content (}\mu l \times mg^{-1})}$$

Effects of intra- and extracellular taurine on ion fluxes in astrocytes

One of the goals of this study was to discriminate between intracellular and extracellular taurine effects on astrocyte ion fluxes. Extracellular effects were simulated in the following way: after 5 h preincubation in salt solution, the cells were exposed to 1 mM taurine for one min before ion flux measurements were taken. This concentration of taurine was maintained throughout the measurement period. Intracellular effects were simulated by incubating the cells with salt solution with 1 mM taurine in order to load them to 60 mM (taurine loaded cells). Extracellular taurine concentration was kept at 1 mM throughout the measurement period.

Potassium. Figure 3 represents the results of taurine effects on the K^+ system. Neither extra- nor intracellular taurine had an effect on the K^+ content of astrocytes. As well, the unidirectional influx of K^+ did not show any difference to controls if taurine was present only extracellular or the cells were loaded with taurine. In a third series of K^+ experiments the net accumulation of K^+ into astrocytes was measured by suddenly exposing cells equilibrated with 3 mM extracellular K^+ to 12 mM K^+ for 1 min. Normally astrocytes gain about 500 nmol K^+ per mg protein during this minute and this increase is carrier-mediated and represents 70–80% of their resting content at 3 mM K^+. Extracellular exposure to taurine did not significantly change the net accumulation of K^+, however, loading with intracellular taurine decreases the K^+ accumulation by half (see Fig. 3).

Sodium. The unidirectional flux of Na^+ was measured in control cells (5 h in salt solution without taurine) as 296.4 ± 66.0 nmol × mg^{-1} × min^{-1} (n = 5). Cells exposed to extracellular taurine had a reduced Na^+ influx at 194.9 ± 54.1 nmol × mg^{-1} × min^{-1} (n = 5), however, this effect was not significant. Intracellular taurine (loaded cells) showed no change in Na^+ flux (312.9 ± 78.5 nmol × mg^{-1} × min^{-1}).

Chloride. The Cl^- influx was determined to be 28.5 ± 3.3 nmol × mg^{-1} × min^{-1} (n = 5). This influx was

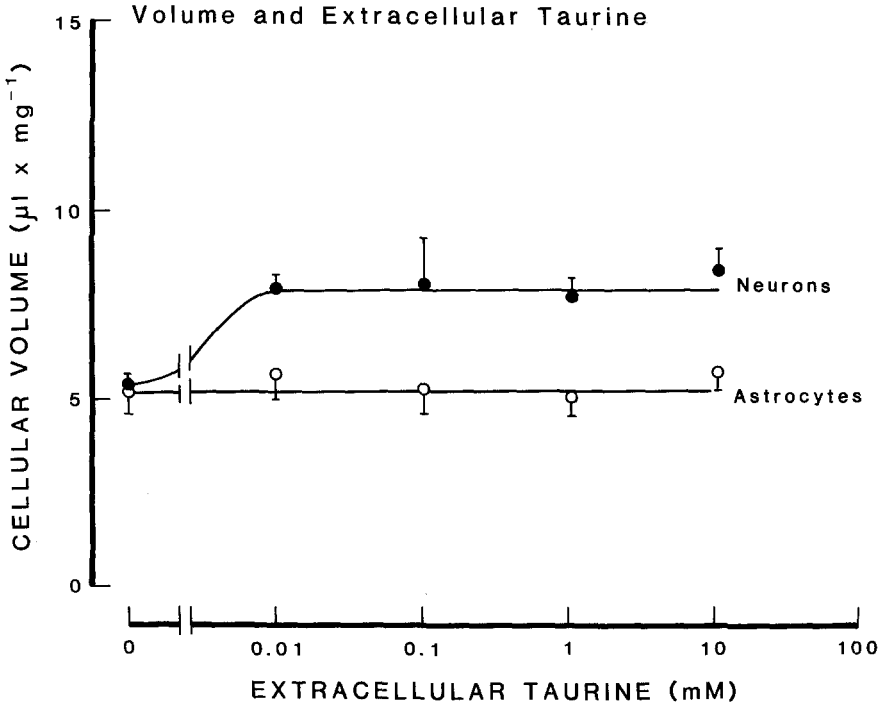

Fig. 2. Volume of neurons and astrocytes after 5 h of exposure to different extracellular concentrations of taurine (in µl cellular water per mg cellular protein; n = 5; S.E.M. indicated)

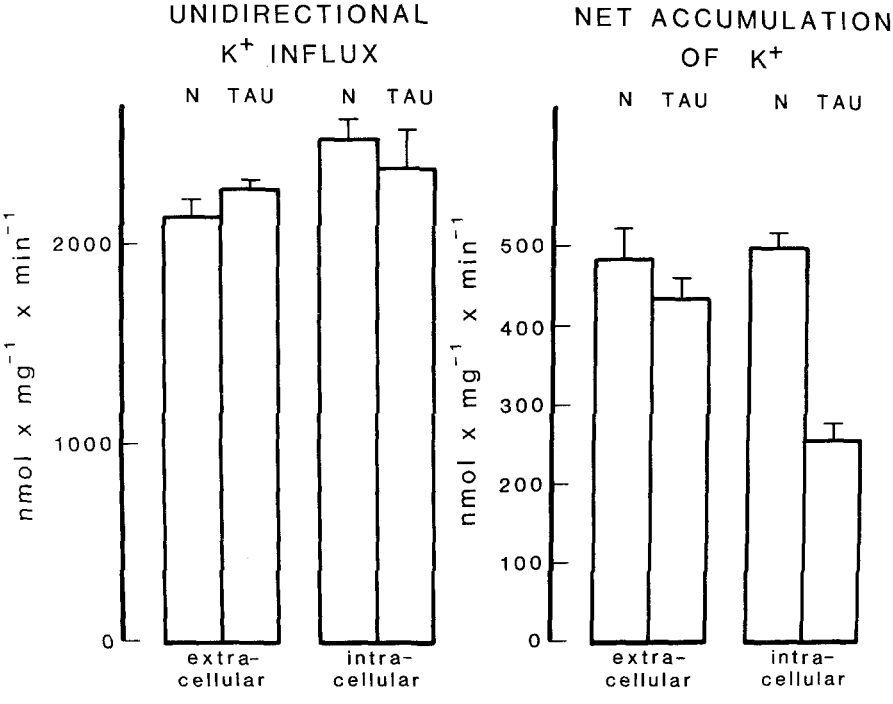

Fig. 3. *Left*: The effects of intra- and extracellular taurine on the astrocytic accumulation of K^+ in response to high extracellular K^+ (12 mM). *Right*: The effect of intra- and extracellular taurine on unidirectional influx of K^+ in astrocytes. N = Normal, TAU = taurine presence; n = 5, S.E.M. are indicated

increased to 33.9 ± 7.4 nmol × mg^{-1} × min^{-1} by extracellular exposure to taurine. However, this is not a significant increase over control values. Taurine loading (intracellular taurine) also did not alter the influx of Cl^- (29.0 ± 4.1 nmol × mg^{-1} × min^{-1}).

Calcium. Exposure to extracellular taurine decreased the influx rate of Ca^{2+} by approximately one third (see Fig. 4), however, this effect was not significant. In contrast, the Ca^{2+} influx into taurine loaded astrocytes was doubled. This effect of intracellular

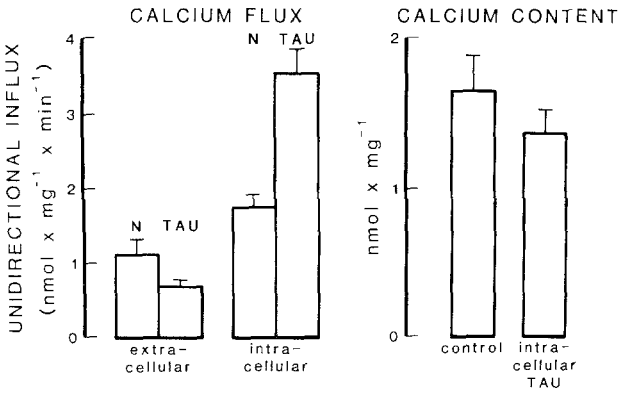

Fig. 4. Effects of intra- and extracellular taurine on calcium unidirectional influx and content. Meaning of symbols are the same as for Fig. 3

taurine was significant $p \ll 0.05$, using the t-test. However, in the same situation, taurine-loaded cells, there were no significant changes in the total Ca^{2+} content of the astrocytes (see Fig. 4).

Discussion

Taurine transport

There is now ample evidence that cultured neurons and astrocytes have a saturable sodium-dependent high affinity uptake and a low affinity uptake for taurine (for review see Oja and Kontro 1983). The cortical mouse astrocytes and neurons used in this study showed similar characteristics: an uptake with a velocity of 1–2 nmol × mg^{-1} × min^{-1} at 10 μM taurine which is saturable and an uptake at concentrations > 100 μM with higher velocities (Table 1). Therefore, the cultured cells used seem to behave "normally" with respect to taurine transport kinetics and can be used as models for taurine effects on ion and water transport. It was demonstrated earlier that these mouse astrocytes are an adequate model for glial cells in vivo with respect to ion transport and electrical characteristics (Walz 1986).

Taurine compartments

Endogenous analysis of the taurine content showed that cultured astrocytes and neurons contain taurine in intracellular concentrations of 10–20 mM. If the cells had been exposed for 5 h to taurine free salt solution, there was no significant change observed in these taurine concentrations. This means at these intracellular "normal" taurine levels, taurine metabolism is non-existent or very low. When the salt solution contained 1 mM taurine during this 5 h incubation, taurine was taken up by both cell types. During this exposure the endogenous taurine content went up threefold in astrocytes and doubled in neurons. In astrocytes, the amount of radioactivity accumulated from extracellular taurine (522 nmol × mg^{-1}) corresponds to the amount of taurine the cells (510 nmol × mg^{-1}) would accumulate over 5 h with the initial velocity of 1.7 nmol × mg^{-1} × min^{-1}. However, only about half of it can be detected as taurine (this corresponds to an intracellular concentration of 60 mM). The other half must be metabolized into (an) other substance(s) which is present in the cells. The accumulated taurine and its metabolites amount to a concentration of at least 120 mM, *if* dissolved in the cytoplasma. For neurons the analysis is different, from the original velocity an accumulation of 1650 nmol × mg^{-1} over 5 h can be calculated. However, the radioactivity accumulated corresponds to 773 nmol × mg^{-1}, of which only 20% is in the form of taurine (Table 2). Either the initial velocity of taurine uptake is not maintained or taurine metabolites are subsequently lost into the surrounding salt solution. In any event the taurine content of neurons only doubles. This suggests that neurons have a less developed capacity to store taurine than do glial cells.

Taurine action on water spaces

Extracellular taurine in various concentrations had no effect on neuronal or astrocytic water content over a short-term period (up to 10 min; results not shown). However, *extracellular* taurine (10 μM) increased the water content of neurons by 30% (Fig. 2). Further increases of the taurine concentration had no effect on cell water content. Loading glial cells with taurine to concentration up to 60 mM had no effect. The effect of low levels of extracellular taurine on neurons might be based on receptor activation rather than a direct increase of intracellular osmolarity by taurine accumulation. Through this mechanism taurine might interact with metabolic processes, e.g. calcium movements to alter the osmolarity of the neurons. Alternatively this volume increase may reflect a receptor-mediated change in ion permeabilities activated by long-term exposure to low taurine concentrations. In glial cells, however, it is surprising that the taurine, stored within the cells after loading has no effect on cellular water content. The amount of accumulated taurine results in an increase of the cellular concentration of 40 mM. If taurine metabolites, which are kept in the cells, are

included, the gain in osmolites corresponds an increase of at least 120 mM. This would increase the cellular osmolarity by 13 and 40% respectively. One possibility is that taurine is not completely dissolved in the cytoplasmic solution, because the very high water permeability of astrocytes alone would lead to an immediate compensatory water influx if there were a net gain of osmotically active substances (Walz and Hinks 1985). One assumption is that taurine is sequestered in both, neurons and glial cells. Such a sequestration of taurine has not been demonstrated to date. Astrocytes are not known to contain storage vesicles for small molecules like taurine. An alternative explanation would be a compensatory loss of some other compound or ion from the cells when large amounts of taurine are taken up. Our experiments, however, rule out the possibility that potassium, the major intracellular ion is involved in such an exchange.

Taurine action on glial ion fluxes

Glial cells are considered to be a major osmo- and ion regulator in the brain (Schousboe 1980; Kimelberg 1983; Walz and Hertz 1983b). There is, however, no study available connecting taurine with a proposed function as osmoregulator (Van Gelder and Barbeau 1985) and ion transport properties of glial cells. Short term exposure of glial cells to extracellular taurine in various concentrations had no effect on their water content (results not presented). Extracellular application of taurine has no effect on electrical properties of glial cells (Kettenmann et al. 1984; W. Walz, unpublished observations). Extracellular taurine in a concentration of 1 mM did also not change any of the ion fluxes studied here. This rules out the possibility that taurine, released from neurons has any short-term regulatory effect on glial physiological properties. It has been shown that taurine can be accumulated to tissue concentrations as high as 50 mM and that LRM55 glial cells maintain an intracellular taurine concentration of approximately 30 mM (Voaden et al. 1977; Huxtable 1982; Martin and Shain 1979; Shain and Martin 1984) our astrocytes contain a taurine concentration of about 20 mM. We were therefore interested to determine whether glial cells loaded with a comparable high amount of taurine would change their ion regulatory properties. We chose the concentration of 60 mM (Fig. 3), which corresponds mouse cortex tissue concentrations in vivo. These cells had drastically increased calcium fluxes. The total calcium content did not change. One has therefore to conclude that the increased influx of calcium is due to an increased turnover rate of calcium in- as well as outflux. An interesting question is if the intracellular free cytosalic calcium activity is increased. Only 0.05% of the total intracellular calcium of these cells is in its free cytosolic form (Walz and Wilson 1986), so that a drastically increased calcium turnover of the glial membrane might well increase this free cytoplasmic calcium activity, although we did not measure this. Taurine was shown to modify calcium fluxes across biological membranes of various tissues (see below).

The taurine effect on calcium fluxes may also be the reason for the 50% inhibition of the intracellular potassium increase, evoked by a physiological increase of extracellular potassium. Stimulated potassium uptake is one of the major functions of glial cells known to date. The main enzyme in this reaction is the Na^+, K^+-ATPase. It exchanges sodium and potassium and provides the driving force (via the sodium gradient) for the NaCl/KCl carrier (Walz and Hinks 1986). If intracellular taurine is changing free cytoplasmic levels of calcium, one would expect the activity of the Na^+, K^+-ATPase to be affected: increased intracellular calcium was shown to inhibit the enzyme function by increasing the proportion of the K^+-K^+ self exchange the enzyme facilitates (Trachtenberg et al. 1981). Such a K^+-K^+ self exchange is physiologically unimportant and would not be detected under the experimental conditions used. It is somewhat surprising that under these conditions taurine does not affect channel-mediated K^+ fluxes which are responsible for spatial buffer currents in glial cells (Walz 1986). However, in mouse astrocytes these intense K^+ fluxes are mediated by K^+ inward rectifier channels which are not sensitive to calcium (Walz and Hinks 1987).

Taurine actions on neuronal ion transport

There is lack of studies on glial ion transport, however, several studies have been carried out to find the basis of the very well known depressant action of taurine on neuronal activity. The work of Okamoto et al. (1983a, b) showed that extracellular taurine increases chloride conductivity and decreases calcium conductivity of cerebellar Purkinje cells. The same was found for hippocampal neurons (Zeise 1985). The opening of chloride channels is quite obviously a direct cause for the depressant activity of taurine.

Taurine and calcium

Several studies have now shown that taurine interacts with calcium in neuronal and muscle tissue (reviewed

in Pasantes-Morales et al. 1982) and in glial cells. However, the concentrations of taurine used were very high, varying from 10–25 mM *extracellularly* in the studies in excitable tissue to 60 mM *intracellularly* as used in this study. Taurine increases the phosphate-dependent calcium uptake via the Ca^{2+} pump into brain synaptosomes and isolated rod outer segments (Pasantes-Morales et al. 1979, 1982; Pasantes-Morales and Gamboa 1980). However, it was also found that another calcium entry mechanism, probably via channels, was stimulated by taurine. In astrocytes the total calcium influx is via channels (Walz and Wilson 1986). There is evidence that the main function of taurine could be the modulation of calcium transport, although the circumstances of this modulation (source of taurine, intra- or extracellular action or both) are not yet clear.

Conclusions

The proposed mechanisms of taurine function as an ion regulator or osmoregulator are highly speculative, there is a general lack of supporting evidence for these taurine roles. We conclude that neurons and astrocytes in primary culture respond similarily when exposed to extracellular taurine. Taurine loading does not effect intracellular water content. Glial cell volume is not affected at all by taurine loading, which is consistant with a lack of taurine effect on resting Na^+, K^+ and Cl^- movements. Intracellular taurine (60 mM) did significantly decrease activated K^+ accumulation by the carrier system and doubled the Ca^{2+} entry. Both K^+ and Ca^{2+} compartmental shifts are associated with neurotransmission so that it is reasonable to conclude that intracellular taurine has a role in regulating the ion homeostasis during neurotransmission. Ion shifts between neurons and glial cells during neurotransmission are associated with water movements. Any interference of taurine with these shifts has to influence water movements associated with neurotransmission (Walz and Hinks 1986). It appears that the effects of taurine on astrocytes are not due to its transient appearance in the extracellular space during neuronal activity but rather to an *increase in intracellular concentration*. Taurine may be a use dependent long-term signal. Glial cells which are situated near well used neuronal pathways are exposed to, and accumulate taurine. This transiently stored taurine has the ability to change Ca^{2+} and K^+ regulatory properties of astrocytes. Glial cells neighboring less well used pathways would have different K^+ and Ca^{2+} metabolism because they do not receive the taurine signal. Observations that activation of β-adrenergic receptors on astrocytes result in release of intracellularly stored taurine (Shain et al. 1986) fit in this picture, indicating that the content of intracellular taurine in glial cells is under control of a set of mechanisms involved in neurotransmission.

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