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Role of taurine in osmoregulation in brain cells: Mechanisms and functional implications

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Summary. All cells including neurons and glial cells are able to keep their volume within a very limited range. The volume regulatory mechanism involves changes in the concentration of osmolytes of which taurine appears to be of particular importance in brain cells. Swelling in brain cells may occur as a result of depolarization or small fluctuations in osmolarity. In isolated brain cells these conditions will always lead to a release of taurine, the time course of which is superimposable on that of the volume regulatory decrease which follows the initial cell swelling. The mechanism responsible for taurine release associated with swelling has not been fully elucidated but a large body of evidence seems to exclude participation of the taurine high affinity carrier. Using a number of inhibitors of anion exchangers it has been demonstrated that both volume regulation and taurine release in brain cells are inhibited by these drugs, implicating an anion channel in the process. It has been a controversial issue as to whether or not taurine release is Ca^{++} dependent. Recent evidence appears to suggest that the release process is not associated with Ca^{++} or Ca^{++} channels. It is, however, quite possible that the swelling process may involve the Ca^{++} calmodulin system or other second messengers. Taurine also contributes to volume regulation after shrinkage of brain cells, in this case by increasing its intracellular concentration. This change is accomplished by an upregulation of the Na^{+}/taurine cotransporter, together with reduced passive fluxes and increased endogenous synthesis.

Keywords: Amino acids – Taurine – Swelling – Astrocytes – Neurons

Introduction

In order to maintain the dynamic equilibrium of the intracellular milieu, cells must be able to keep their volume within a very limited range. Small changes in the extracellular osmolarity as well as in the osmotic gradient resulting from

transmembrane transport processes may, however, occur during normal cell activity. Therefore cells must possess mechanisms allowing effective adjustment of volume in accordance with such changes in osmolarity (Macknight, 1988). This may be the reason why cell volume regulation appears to be a fundamental property of animal cells conserved throughout evolution (Chamberlain and Strange, 1989). Brain cells are no exception and volume regulatory mechanisms have been observed in neurons as well as in glial cells (Kimelberg and Ransom, 1986; Pasantes-Morales and Schousboe, 1988; Pasantes-Morales and Martin del Rio, 1990; Pasantes-Morales et al., 1993). A detailed knowledge of the mechanisms underlying this process in brain cells may be of particular importance due to the limits of expansion of the brain volume imposed by the skull. As a consequence, generalized brain edema occurring in relation to trauma, hyponatremia, hepatic encephalopathy as well as more localized changes in cell volume as those occurring in concert with hyperexcitable states such as epilepsy and energy failure, may have fatal outcomes (Kimelberg and Ransom, 1986; Trachtman, 1992).

Cell volume regulation is accomplished by activation of transmembrane fluxes of osmolytes in the direction necessary to reinstate proper volume. A relatively limited number of molecules seem to serve the function as osmolytes. The molecules may be either inorganic ions, mainly sodium, potassium and chloride or organic compounds such as amino acids, polyalcohols and amines (Thurston et al., 1980; Holopainen et al., 1986; Levi and Patrizio, 1992; Patel and Hunt, 1985; Sanchez-Olea et al., 1993a; Pasantes-Morales et al., 1994a,b,c; Strange et al., 1993, 1994; Isaaks et al., 1994). Among these substances taurine has been proposed to play an important role (Pasantes-Morales and Schousboe, 1988). Taurine, as other organic osmolytes, is a relatively inert molecule, which even at high concentrations is not only compatible with the function and organization of macromolecules, but may in addition act as an osmoprotectant counteracting the deleterious effects of denaturating solutes as inorganic ions. Besides, since taurine is not involved in metabolic reactions, including protein synthesis, it may be transported to adjust osmotic disturbances, outside and inside the cell and through intracellular compartments, without imposing further metabolic stress on the cell. The present review will be focused on a discussion of the present state of knowledge about the mechanisms by which taurine contributes to the volume regulatory process in brain cells.

Taurine fluxes and cell volume regulation

I. Cell swelling and taurine efflux

Brain cell volume regulation has been studied mainly in relation to changes in cell volume caused by anisosmotic conditions (Kimelberg and Frangakis, 1986; Pasantes-Morales and Martin del Rio, 1990). Taurine is a major component of the organic osmolyte pool which plays an important role in the resistance of the brain to severe hyponatremia. During hyponatremia, the

concentration of organic osmolytes is modified according to the osmotic gradient, thus maintaining normal levels of cerebral hydration. Taurine importantly contributes to this adaptive response and, in fact, the largest changes in concentrations observed among the organic osmolytes, are those exhibited by taurine.

In the rat and mouse brain, taurine levels decrease about 40%–60% after 3 days of hyponatremia and after 14 days, the taurine pool is essentially depleted (Thurston et al., 1987; Verbalis and Gullans, 1991). Also in more physiological conditions, as during the mild decrease in plasma osmolarity occurring during pregnancy, a marked fall in brain taurine content has been reported (Law, 1989).

Swelling associated taurine release can also be observed *in vivo* using the microdialysis technique. Thus, perfusing the brain with a hypotonic solution, the extracellular taurine level is increased (Solis et al., 1988; Wade et al., 1988; Lehmann, 1989). It has also been repeatedly demonstrated that during energy failure due to ischemia or hypoxia there is an increased extracellular level of taurine (Benveniste et al., 1984; Hagberg et al., 1985). This increase in release of taurine can best be explained as being triggered by the cell swelling associated with this condition and induced by the increase in extracellular K^+ related to energy failure (Hansen, 1985). These findings underline the contribution of taurine, together with other organic osmolytes, in the maintenance of brain cell volume in physiological conditions, as well as in pathological states.

Non anisosmotic cellular edema occurs in brain in association with numerous pathologies, including epilepsies, ischemia, or after administration of excitatory amino acids (McManus and Churchwell, 1994). In all these conditions, the efflux of taurine from brain is enhanced, possibly as a consequence of the cell swelling (Lehmann, 1990). A decrease in taurine content has been reported concomitant with the development of brain edema in hepatic encephalopathy (Hilgier and Olson, 1994).

The mechanism of taurine efflux associated with swelling has been investigated in cultures of either neurons or glial cells since these are convenient experimental preparations, as problems with complexity, heterogeneity and inaccessibility are circumvented. When cultured neurons and astrocytes are exposed to solutions of decreased osmolarity they rapidly swell and subsequently an active regulatory process is initiated allowing the cells within a few minutes to partly regain their original volume even when the anisosmotic condition persists (Kimelberg and Frangakis, 1986). This volume regulatory process is accomplished by a rapid efflux of intracellular osmolytes, of which taurine is a prominent component (Pasantes-Morales and Schousboe, 1988). Figure 1 illustrates that the time course of taurine release in astrocytes closely follows that of the volume regulatory decrease. The taurine release that occurs during exposure to a 50% hyposmotic solution (160 mOsm) accounts for 80% of the intracellular taurine content in astrocytes or neurons and the amount released is proportional to the reduction in osmolarity. Taurine release can be reliably detected with reductions in osmolarity as low as 10% (Pasantes-Morales and Schousboe, 1989; Pasantes-Morales et al., 1990).

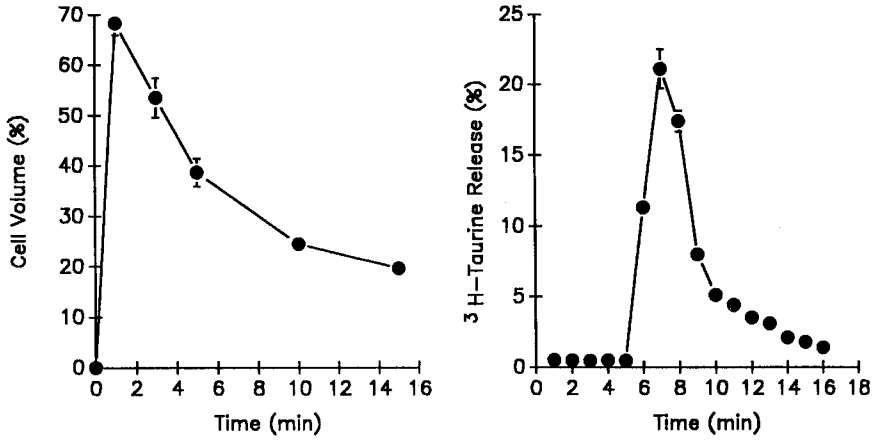

Fig. 1. The effect of hyposmotic conditions on cell volume (left panel) and taurine release (right panel) of cultured astrocytes. Cells cultured in 24-well multitest dishes were washed with HEPES-buffered Eagle's medium and then incubated in glucose-free hyposmotic medium (150 mOsm, 55 mM NaCl). Cell volume was determined after 0, 1, 3, 5, 10 and 15 min, following the distribution of ^{14}C-3-0-methyl glucose (see Pasantes-Morales and Schousboe, 1988). Results are means ± SEM (vertical bars, when extending the symbols) of 8 experiments. For measurements of taurine release, cells were preincubated with ^{3}H-taurine (5 μM, 1.3 μCi/culture dish), washed and superfused with Krebs-HEPES medium (2 ml/min) at 37°C. After 15 min of superfusion (wash) samples were collected every 1 min. During the time period 5–16 min, the superfusion medium was replaced by an analogous medium with reduced osmolarity (150 mOsm, 55 mM NaCl). At the end of the superfusion, radioactivity in samples and that remaining in cells was determined by liquid scintillation spectrometry. Results are expressed as fractional release, i.e., the radioactivity in fractions as percent of total radioactivity in the cells at start of the superfusion, excluding the washing period. The figure represents the average efflux pattern of ^{3}H-taurine release from 8 experiments with SEM values shown by vertical bars when extending beyond the symbols

II. Taurine and brain regulatory volume increase

Taurine is part of the organic osmolyte pool which contributes to brain adjustment to hyperosmotic situations. An increase of brain taurine content is consistently observed in chronic hypernatremic mice and rats (Thurston et al., 1980; Nieminen et al., 1988). Large increases of brain taurine are also observed during chronic hyperglycemia (Tratchman et al., 1991).

The mechanism of taurine accumulation subsequent to cell shrinkage has been investigated in glial cells in culture. As occurring *in vivo*, and in contrast to the rapid response observed upon cell swelling, a significant increase of taurine content in cells exposed to hyperosmotic solutions occurs only after 10–12 h (Sánchez-Olea et al., 1992; Beetsch and Olson, 1996). After 24 h, the initial concentration of taurine in astrocytes was increased by 80%–100%. The Na^{+}-dependent carrier seems clearly involved in the accumulation of taurine associated with the cell adaptation to the volume change (Fig. 2), since a significant increase in the V_{max} (36%–70%), but no change in the K_m has been observed (Sánchez-Olea et al., 1992; Beetsch and Olson, 1996). The mechanism of this upregulation of the taurine transporter is unclear at

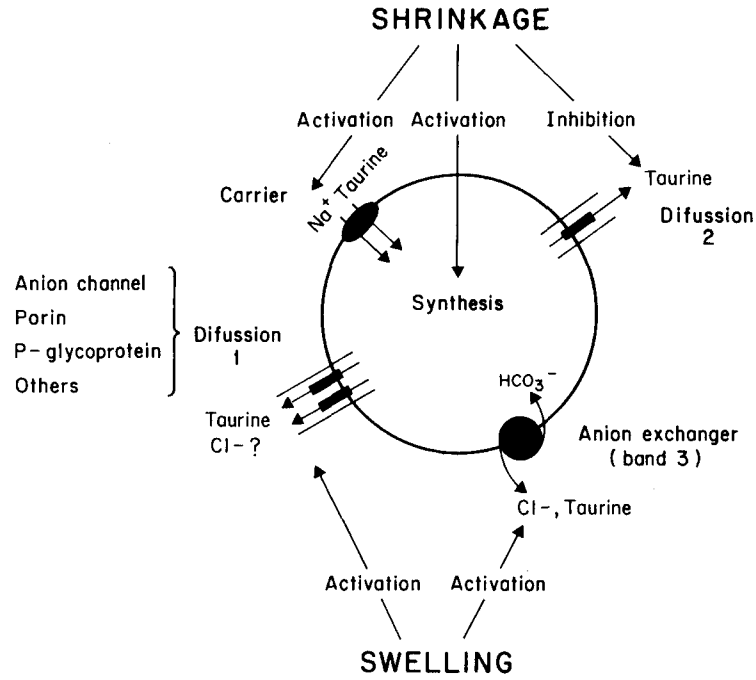

Fig. 2. Possible mechanisms responsible for the adaptive changes in taurine concentration subsequent to increases or decreases in cell volume

present. Reductions in the passive efflux rate and possibly an enhanced rate of synthesis also contribute to the increase in cell taurine levels. An increase in taurine transport by isolated nerve endings has been reported associated with chronic hypernatremia. Apparently, chronic hyperglycemia activates the same brain cell volume regulatory adaptation, including an increase in taurine transport (Tratchman et al., 1992).

It has been proposed that taurine acts as a cerebral osmoprotective molecule in chronic hypernatremic dehydration. This is supported by the higher susceptibility to hypernatremia exhibited by taurine deficient postweanling kittens, expressed by higher mortality and seizure activity (Tratchman et al., 1988).

Mechanism of taurine efflux

Swelling associated taurine efflux is sodium and temperature independent, suggesting possible elimination of the Na^+/taurine cotransporter as the mechanism responsible for this release (Pasantes-Morales et al., 1990). This is further supported by the demonstration that changes in osmolarity do not affect the kinetics of the saturable component of taurine transport whereas the non-saturable component is increased under these conditions (Sánchez-Olea et al., 1991; Schousboe et al., 1991). The involvement of a diffusional mechanism (Fig. 2) is underlined by experiments showing that the direction of taurine flux through the pathway activated by swelling is determined exclu-

sively by the concentration gradient (Sánchez-Olea et al., 1991). Thus, under physiological conditions when the intracellular taurine concentration is much higher than the extracellular level, swelling will result in taurine efflux whereas if the gradient is reversed influx will occur. This phenomenon has been observed *in vivo* during experimental hyponatremia leading to a transfer of taurine from neurons containing large amounts of taurine to astrocytes which *in vivo* contain much less taurine than neurons (Ottersen, 1988; Nagelhus et al., 1993). This may constitute an important mechanism by which astrocytes are able to protect neurons from swelling.

The nature of the diffusional pathway

While it has been established that swelling activated taurine release is a diffusional process, the identity of the pore has yet to be determined (Fig. 2). The suggestion that this could be an anion channel-like structure comes from the observation that chloride channel blockers such as NPPB (5-nitro-2-(3-phenylpropylamino)-benzoic acid) or DDF (1,9-dideoxyforskoline) can fully inhibit the regulatory volume decrease (Jackson and Strange, 1993; Sánchez-Olea et al., 1993b). This observation raised the possibility that osmolytes other than chloride should be sensitive to these blockers. This has been found to be the case for taurine efflux, which is markedly inhibited by NPPB and DDF (Sánchez-Olea et al., 1996) and for inositol efflux, which also is sensitive to these drugs (Gonzáles et al., 1995). Other potent inhibitors of chloride channels, such as polyunsaturated fatty acids, also inhibit taurine release and consequently the regulatory volume decrease (Sánchez-Olea et al., 1995a). More direct evidence to support this notion comes from electrophysiological identification of a swelling activated anion channel in astrocytes carrying taurine with a P_{Tau}/P_{Cl} ratio of 0.4 (Jackson and Strange, 1993; Roy, 1995). This channel is sensitive to all of the drugs which block regulatory volume decrease and taurine efflux.

A number of chloride channels activated by cell swelling have recently been cloned and at least one of these (ClC2) is present in the brain (Paulmichl et al., 1993). Another possible candidate for an anion channel-like molecule capable of transporting taurine is a 72 amino acid peptide, which forms a channel permeable to large anions and which may have a pore dimension of 8 A (Moorman et al., 1995). In this channel, taurine is the most permeable molecule having a P_{Tau}/P_{Cl} ratio of 70. The presence of this peptide in brain tissue remains, however, to be established.

In addition to chloride channels as such, other channel forming molecules could be considered as possible pathways for taurine efflux. These are (Fig. 2) the band 3 type anion exchanger, the P-glycoproteins and the porins (Goldstein and Brill, 1991; Valverde et al., 1992; Liu and Colombini, 1992). The suggestion that band 3 may be involved came from the finding that the classical inhibitors of anion exchangers (Cabantchik and Greger, 1992) DIDS (4,4′-diisothio-cyanostilbene-2,2′-disulfonate) and niflumic acid decrease swelling activated taurine release (Pasantes-Morales and Schousboe, 1989;

Sánchez-Olea et al., 1996). Moreover, an anion exchanger of the erythrocyte band 3 type is present in brain and its chloride channel component is sensitive to NPPB and DDF (Kay et al., 1991). The P-glycoprotein is a member of the ATP binding cassette superfamily which is responsible for the multidrug resistance of tumor cells (Higgins, 1995). This drug resistance is based on the ability of the P-glycoprotein to extrude drugs. However, when this ability is not required, the molecule may function as a swelling activated chloride channel which can be blocked by NPPB, DDF and nifedipine (Valverde et al., 1992). All of these compounds are inhibitors of the swelling induced solute efflux including taurine efflux (Sánchez-Olea et al., 1995b, 1996). Although P-glycoprotein is expressed not only in tumor cells but in a number of normal cells (Higgins, 1995), its presence in the brain has not been established.

The porins are channel forming proteins present in lymphocyte cell membranes as well as in the outer mitochondrial membrane, which allow passage of numerous metabolites (Liu and Colombini, 1992). These porins may also function as chloride channels. Importantly, a molecule identical to lymphocyte porin has been cloned from brain and shown to function as a swelling activated chloride channel when expressed in astrocytes (Dermietzel et al., 1994).

It should be emphasized that yet unidentified molecules could be involved and that the functional importance of any of the above mentioned molecules in swelling induced taurine efflux remains to be firmly established. It may be of interest that phenylsuccinate which is a classical inhibitor of the mitochondrial dicarboxylate carrier (Passarella et al., 1987) has been found to inhibit swelling induced taurine release in the brain and in cultured neurons and astrocytes (Bruhn et al., 1996). Whether or not molecules related to this mitochondrial membrane associated transporter could be involved in swelling induced taurine flux requires further investigation to be elucidated.

Depolarization induced swelling and taurine efflux

The most frequently used conditions to chemically depolarize brain cells are exposure to either elevated K^+ concentrations or to excitatory amino acids (EAAs). These conditions also lead to cell swelling (Hertz and Schousboe, 1975). In the case of K^+ depolarization, swelling is secondary to the uptake of K^+ or Cl^- and can be prevented by maintaining the $K^+ \times Cl^-$ product constant (Pasantes-Morales and Schousboe, 1989). In case of EAAs, the exact mechanism for swelling has not been fully elucidated but it appears to involve activation of ionotropic and metabotropic glutamate receptors as well as the electrogenic glutamate transporters (Koyama et al., 1994; Bender et al., 1995). Analogous to swelling induced by hyposmotic solutions, swelling induced by exposure to K^+ or EAAs will result in taurine efflux in both neurons and astrocytes (Pasantes-Morales and Schousboe, 1989; Schousboe et al., 1991; Koyama et al., 1994). In keeping with the fact that K^+ induced swelling is Cl^--dependent, taurine release associated with K^+ is inhibited by removal of Cl^- (Pasantes-Morales and Schousboe, 1989). In this context it should be men-

tioned that taurine release is reduced by DIDS (Pasantes-Morales et al., 1990). It is, however, unclear whether this effect of DIDS is exerted on K^+/Cl^- uptake or the taurine efflux mechanism directly. Swelling and taurine efflux resulting from exposure of brain cells to EAAs may at least partly be reduced by EAA receptor antagonists. However, due to the fact that glutamate transporters may play an important role, this issue has not yet been fully elucidated (Koyama et al., 1994; Bender et al., 1995).

Role of Ca^{++} and other second messengers in taurine efflux

It was originally claimed that K^+ stimulated taurine release from brain tissue was a Ca^{++} dependent process, analogous to release of classical neurotransmitters such as glutamate and GABA (Oja and Kontro, 1983). This view has been subsequently challenged by using experimental conditions not causing unspecific membrane permeability changes and also by the availability of more specific blockers of voltage gated Ca^{++} channels responsible for depolarization coupled release of neurotransmitters (Schousboe et al., 1990; Sánchez-Olea et al., 1995b). Since swelling induced taurine release is clearly independent of Ca^{++} (Pasantes-Morales et al., 1990) and since the majority of K^+ induced taurine release is associated with swelling (Pasantes-Morales and Schousboe, 1989; Schousboe and Pasantes-Morales, 1989), it is not surprising that K^+ induced taurine release has been found to be essentially Ca^{++} independent. In fact, swelling induced release of taurine observed under hyposmotic conditions is unaffected by Ca^{++} free media or by EGTA and BAPTA and is insensitive to verapamil and diltiazem. However, it was found to be inhibited by dihydropyridines but in a Ca^{++} independent manner excluding the possibility that the dihydropyridines exert this action via voltage gated Ca^{++} channels (Sánchez-Olea et al., 1995b).

So far there is no information available on the involvement of other second messenger systems in swelling induced taurine release, except for a demonstration in brain slices that taurine efflux is sensitive to inhibitors of the Ca^{++} calmodulin system (Law, 1994). It should be mentioned, however, that astrocyte swelling may be related to protein phosphorylation involving second messengers (Bender et al., 1992).

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