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Rapid report

Osmolyte contents of cultured astrocytes grown in hypoosmotic medium

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Abstract

Primary rat cerebral astrocyte cultures were grown for 2 weeks in isoosmotic medium (305 mosmol) and then placed in similar medium with a reduced NaCl concentration. During the first hour of growth in this moderately hypoosmotic medium (240 mosmol), the cells lose 88% of their taurine contents, 62% of their alanine contents, and 54% of their aspartate contents while regaining normal volume. Loss of these amino acids accounts for 43% of observed volume regulation. Contents of these amino acids remain decreased during 24 h of growth in hypoosmotic medium. In contrast, potassium, glutamate, glutamine, and asparagine contents are not changed, relative to cells in isoosmotic medium, at time points between 1 h and 24 h of hypoosmotic exposure. The data suggest astrocytes contribute to net loss of amino acids, but not potassium, from brains exposed to hypoosmotic conditions in situ.

Keywords: Potassium; Taurine; Osmotic pressure; Cell volume; Brain edema

The brain is protected from osmotically induced volume changes by various mechanisms. During the first several hours of hyperosmotic treatment (350–400 mosmol), brain tissue accumulates inorganic osmolytes [1,2]. After days of persistent serum hyperosmolality, concentrations of organic compounds including amino acids and sugar polyols increase in brain tissue [3–5]. This accumulation of organic osmolytes is mimicked in cultures of cerebral astrocytes grown in hyperosmotic medium, suggesting astrocytes contribute to the increased content of these compounds in brain [6–9].

Conversely, hypoosmotic hyponatremia (≈240–250 mosmol) is accompanied by loss of inorganic ions from the brain parenchyma within a few hours with little to no change in organic osmolyte content [3,10]. Reduction in the content of amino acids and other organic osmolytes occurs over several days of persistent hypoosmotic exposure [3,10,11]. In contrast to the brain in vivo, cultured astrocytes lose taurine and other amino acids within minutes of a more extreme hypoosmotic exposure (150–170 mosmol) without net loss of inorganic ions [12–14]. These differences between in vivo and in vitro responses to osmotic swelling may be explained if organic osmolytes lost from astrocytes in the hypoosmotic brain are redistributed to the extracellular space or into other cell types. Alternatively, magnitudes of organic and inorganic osmolyte losses from astrocytes may differ between prolonged, moderate hypoosmotic exposure (characteristic of in vivo studies) and short-term but more severe hypoosmotic treatment (typical of culture studies). The purpose of these experiments was to examine changes in osmolyte content of cultured astrocytes during a hypo-

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osmotic exposure which is similar in magnitude and duration to that used in studies of the intact animal.

Pregnant Sprague–Dawley rats were obtained from Harlan (Indianapolis, IN). Newborn calf serum, minimum essential medium in Earle's balanced salts without glutamine (MEM), MEM amino acids (100× concentration), MEM vitamins (50× concentration), and antibiotic solution (5000 U/ml penicillin, 10 mg/ml streptomycin) were obtained from Life Science Technology (Grand Island, NY). All other chemicals were the finest grade available and were obtained from Sigma Chemical Co. (St. Louis, MO).

All procedures were approved by the Laboratory Animal Care and Utilization Committee of Wright State University and conformed to the *Guide for the Care and Use of Laboratory Animals*. Primary astrocyte cultures were prepared and maintained as previously described [6,15]. After 2 weeks in vitro, standard culture medium (305 mosmol) was replaced by experimental culture medium formulated to have an osmolality of 305 mosmol (isoosmotic) or 240 mosmol (hypoosmotic). These media had identical concentrations of amino acids, vitamins, antibiotics, bicarbonate, and calf serum as standard culture medium. Earle's balanced salt solution of the experimental medium was made from laboratory reagents and pyrogen-free distilled water. Hypoosmotic medium was made by reducing the concentration of NaCl. Cells were harvested before or 1 h, 12 h, or 24 h after exposure to experimental culture medium.

Total intracellular volume and contents of potassium and amino acids were measured in each culture dish. At the designated times, culture dishes were rinsed twice with phosphate-buffered saline (PBS) containing 2.7 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 2.7 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1 mM 3-*O*-methylglucose (3-OMG), and sufficient NaCl to match the osmolality to that of the experimental medium (pH 7.3). Cultures then were incubated at 37°C in this same PBS plus 0.5 μCi/ml [^{3}H]3-OMG and 0.5 μCi/ml [^{14}C]sucrose. After 5 min, the cells were rinsed three times with an osmotically matched sucrose solution containing 1 mM phloretin, 10 mM Tris-base, and 0.5 mM $Ca(NO_3)_2$. pH was adjusted to 7.3 using nitric acid. Cells were scraped into 1 ml of 0.6 M $HClO_4$ plus 4 mM CsCl, and the resulting suspension was centrifuged for 1 min at 10 000×*g*. Protein was determined in the cell pellet [16]. Amino acid, potassium, and radioactivity contents were measured in the supernatant using HPLC, atomic absorption spectroscopy, and liquid scintillation spectroscopy, respectively [12]. Cell volume was defined as the 3-OMG space indicated by the content of ^{3}H [17] and was corrected for residual incubation solution using [^{14}C]sucrose as a marker for extracellular space.

The mean ± S.E.M. volume of cells in standard culture medium prior to exposure to experimental medium was 5.3 ± 0.3 μl/(mg protein) and was not altered at any time point after medium change (Fig. 1). Previously, we and others have shown astrocytes swollen by exposure to hypoosmotic conditions ranging from 110 mosmol to 170 mosmol regulate cell volume towards normal within 15–30 min [18–20]. Consistent with these data, we observed complete volume recovery 1 h after the start of exposure to 240 mosmol medium. At each time point, cells incubated in experimental hypoosmotic culture medium had the same volume as cells incubated in experimental isoosmotic culture medium.

Intracellular potassium concentration, computed by dividing the total cellular potassium content by the total intracellular volume of each culture dish, was similar at each time point for astrocytes incubated in experimental isoosmotic culture medium. The mean ± S.E.M. potassium concentrations averaged for all cultures in isoosmotic conditions was 140.8 ± 4.0 mM (n = 40). In the osmotically swollen brain, loss of sodium and chloride may be the result of transport to the CSF [10] as extracellular volume is reduced [21]. However, potassium is likely to be lost from intracellular compartments. Similar to our previous reports [12,13], we found no loss of potassium from cultured astrocytes during the first hour of hypoosmotic exposure (data not shown). Thus, intracellular potassium concentration returns to its initial value coincident with the return to normal cellular volume. Astrocyte potassium content per mg protein and total protein content of the culture dishes were not altered at any measured time point during exposure to hypoosmotic culture medium.

Contents of taurine, alanine, and aspartate decreased by 88%, 62%, and 54%, respectively within 1 h of hypoosmotic exposure and remained at this diminished level during the 24 h exposure to experimental hypoosmotic culture medium (Fig. 2). The

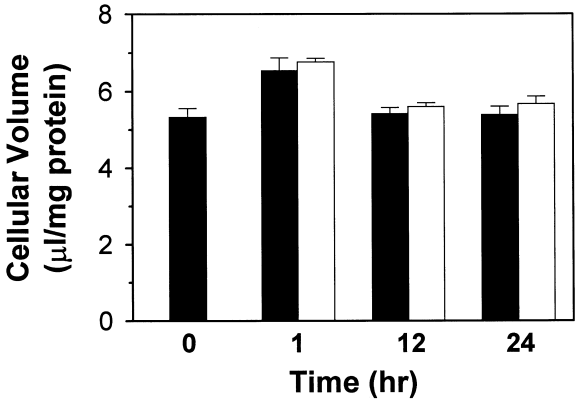

Fig. 1. Cellular volume of astrocytes in isoosmotic medium (solid bars) and hypoosmotic medium (open bars). The time since growth medium was changed to experimental medium is shown on the abscissa. Values shown are the mean ± S.E.M. of 7–14 independent determinations. Volumes of cells in isoosmotic medium were not different at any time point (ANOVA, $P = 0.13$). Cells in hypoosmotic medium had similar volumes as cells in isoosmotic medium at each time point (Student's t-test, $P > 0.38$).

reduction of taurine contents represented a mean ± S.E.M. change in concentration from 26 mM prior to exposure to experimental medium to 2 mM at the 1 h time point. Reduction in taurine content accounted for at least 80% of the total amino acids lost at each time point. Together, taurine, alanine, and aspartate contributed 28 mosmol to the loss of intracellular osmolytes. In contrast, asparagine, glutamine, and glutamate contents were not altered by exposure to hypoosmotic experimental culture medium at any time point. Previous reports have documented glutamate loss from cultured astrocytes during hypoosmotic exposure [22]. The lack of change in glutamate contents in the present studies may be due to the moderate hypoosmotic insult used and the ability of the cells to replenish lost glutamate by metabolism or transport from experimental culture medium during the prolonged time course of these experiments.

The combined loss of taurine, aspartate, and alanine is equivalent to 43% of that required to regain normal cell volume caused by the experimental decrease in extracellular osmolality from 305 mosmol to 240 mosmol. Taurine contributes to the bulk of this osmolality loss, accounting for more than one-third of the total osmolytes needed for complete volume recovery. Neutral osmolytes such as polyols [23] and ions other than potassium also may leave the astrocytes and contribute to the complete volume regulation observed in these experiments. The amino acids measured in these studies may leave the cells as charged or uncharged species. If taurine leaves as an anion, it must abandon a proton in the cell interior, thus lowering the intracellular pH. By comparing intracellular acidification of astrocytes during hypoosmotic (200 mosmol) volume regulation with total

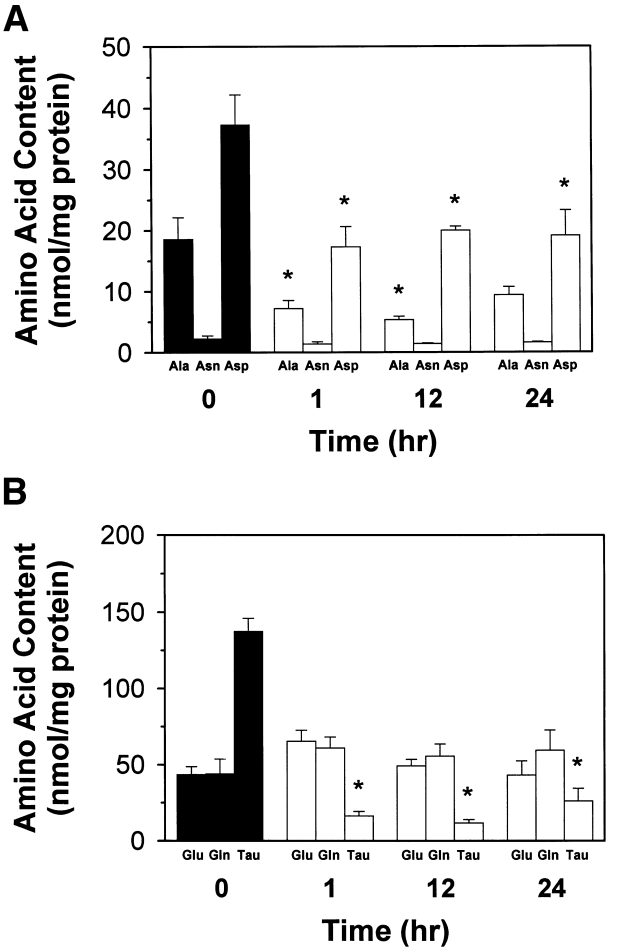

Fig. 2. Amino acid contents of astrocytes in isoosmotic medium (solid bars) and hypoosmotic medium (open bars). The time since growth medium was changed to experimental medium is shown on the abscissa. (A) Astrocyte contents of alanine (Ala), asparagine (Asn), and aspartate (Asp). (B) Astrocyte contents of glutamate (Glu), glutamine (Gln), and taurine (Tau). Notice the different ordinate scales in A and B. Values shown are the mean ± S.E.M. of 7–14 independent determinations. Asterisk indicates mean values significantly lower than that measured in isoosmotic culture medium prior to exposure to hypoosmotic experimental medium ($P < 0.05$ by ANOVA and post hoc Dunnett's test).

taurine loss, we estimated one-third of the taurine leaves the cell as an anion [24]. Efflux of an obligatory positive counter ion to the taurine anion then would contribute to the volume regulatory response; however, the identity of counterions to this loss of anionic taurine is unknown.

Net taurine loss over the time span of these studies may be due to increased unidirectional efflux or decreased unidirectional influx. Taurine is thought to leave astrocytes through hypoosmotic activation of a passive, diffusion pathway [25], possibly membrane anion channels [26]. In contrast, osmotic swelling of astrocytes does not immediately alter the carrier-mediated mechanism of taurine accumulation [25]. Since we found that contents of taurine and other amino acids remain low during prolonged hypoosmotic exposure, either the taurine efflux pathway remains activated despite a return to normal cell volume or the rate of taurine accumulation is diminished by prolonged hypoosmotic exposure.

Hypoosmotic exposure in vivo results in loss of organic osmolytes, including amino acids, only after several hours [3,11]. In contrast, astrocytes in culture conditions rapidly lose taurine and other amino acids following hypoosmotic exposure [7,14]. From the present data, these cells do not reaccumulate taurine, alanine, and aspartate for at least 24 h. The rapid efflux of taurine from astrocytes is consistent with elevations of extracellular taurine concentrations measured during hypoosmotic conditions in vivo [27–29]. This elevated extracelluar taurine may decrease the rate of further taurine efflux and increase the rate of taurine influx by astrocytes. Since transport of taurine from blood to brain is not altered during hypoosmotic hyponatremia [30], enhanced unidirectional efflux at the blood–brain barrier due to elevated extracellular concentrations may lead to a gradual net loss of taurine from the brain over several hours.

In summary, these in vitro data indicate cerebral astrocytes contribute to the loss of taurine and other amino acids from brains of animals exposed to prolonged hypoosmotic hyponatremia. Since cultured astrocytes do not mobilize potassium in response to hypoosmotic swelling, they are not likely to contribute to potassium loss from the osmotically swollen brain. Other cells such as neurons must contribute to the loss of potassium observed in situ. This proposed potassium loss from neurons would lead to membrane depolarization and thus, would be consistent with increases in excitability and seizure susceptibility reported for in vitro [31] and in vivo [32] models of hypoosmotic exposure.

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