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Blood–Brain Barrier Taurine Transport During Osmotic Stress and in Focal Cerebral Ischemia

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Summary: Little is known about blood to brain taurine transport despite substantial evidence suggesting a role of taurine in brain volume regulation during osmotic stress or conditions inducing cell swelling, such as ischemia. We have made measurements of the taurine influx rate constant (K_1) with [^{3}H]taurine in three conditions: raised plasma taurine concentrations induced by infusion with 50 m*M* taurine (10 μl/100 g/min); osmotic stress induced by i.p. injections of 1.5 *M* NaCl (2 ml/100 g) or distilled water (10 ml/100 g); and 4 h of middle cerebral artery occlusion (MCAo). In rats with MCAo, additional determinations were made of tissue water and taurine contents, and blood–brain barrier passive permeability with [^{3}H]α-aminoisobutyric acid. Taurine infusion increased plasma taurine from 110 ± 63 μ*M* (SD) to 407 ± 63 ($p < 0.001$) and decreased taurine K_1 at the blood–brain barrier by 70% ($p < 0.001$), signifying saturable uptake that maintained unidirectional influx constant. Similarly, although hypo- and hyperosmolality increased and decreased plasma taurine concentration, respectively, a reciprocal relationship between K_1 and plasma taurine in these experiments ensured that unidirectional fluxes of taurine into brain were unchanged by osmotic stress. During MCAo, the taurine K_1 was reduced 80% in the ipsilateral ischemic tissue compared with the contralateral nonischemic tissue ($p < 0.001$). This decline may be due to a release of taurine into the brain circulation, because there was a concomitant loss of tissue taurine of 7.4 ± 2.4 mmol/g dry weight ($p < 0.05$). Alternately, if taurine uptake is sodium dependent, the decline might reflect a disruption of the endothelial sodium gradient. **Key Words:** Blood–brain barrier—Taurine—Amino acid transport—Middle cerebral artery occlusion—Volume regulation.

The sulphonic amino acid taurine is abundant in most animal tissues including brain, where it is located in both glial and neuronal fractions. It has been implicated in many biological functions including inhibitory neurotransmission, CNS development, membrane stabilization, and blood pressure regulation (as reviewed in Wright et al., 1986, and Huxtable, 1989). Evidence has also accumulated from a wide range of tissues for a role of taurine in cell volume regulation, with taurine being lost from cells during hypoosmotic stress and gained during hyperosmotic stress (Huxtable, 1992). These changes serve to limit cell swelling and shrinkage, respectively.

Taurine appears to have a similar volume regulatory action in brain. Taurine is lost from brain cells into the extracellular space during hypoosmotic stress (Wade et al., 1988; Solís et al., 1988; Lehmann, 1989). Furthermore, a number of pathophysiological conditions in which cerebral cells swell, such as ischemia–hypoxia, hypoglycemia, or epilepsy, are also accompanied by pronounced increases in extracellular taurine (Shimada et al., 1993; Korf et al., 1988; Tossman et al., 1985; Wade et al., 1987). However, unlike in other tissues, where taurine lost to the extracellular space can diffuse into blood, the brain is separated from the blood by the blood–brain and blood–CSF barriers. Thus, if changes in brain taurine content are involved in total brain as well as brain cell volume regulation, as is the case (Thurston et al. 1983; Trachtman et al., 1988; Verbalis and Gullans, 1991; Sterns et al., 1993), transport at these barrier tissues must play an integral role.

Taurine transport at the blood–brain barrier (BBB) has been investigated in isolated capillaries in vitro, revealing a sodium-dependent uptake pro-

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Abbreviations used: [^{3}H]-AIB, [^{3}H]α-aminoisobutyric acid; BBB, blood–brain barrier; MCAo, middle cerebral artery occlusion.

cess (Tayarani et al., 1989) similar to that observed in glia and neurons (Larsson et al., 1986; Borg et al., 1980). Studies using isolated capillaries probably preferentially allow the examination of carrier systems on the brain facing rather than the blood facing side of the blood–brain barrier (Tayarani et al., 1989; Keep et al., 1993) and thus give information about brain to blood transport. On the other hand, little is known about taurine movement in the opposite direction, its contribution to brain taurine metabolism, or its possible role in cerebral volume regulation.

The present study examines the role of blood to brain taurine transport in brain taurine homeostasis and volume regulation by measuring the influx rate constant for [^{3}H]taurine during changes in plasma taurine concentration, osmotic stress, and middle cerebral artery occlusion (MCAo), a model of focal cerebral ischemia. Tissue taurine concentrations were also measured because the combined information on influx and changes in tissue content allows assessment of efflux. A preliminary report of this work has been given (Stummer et al., 1994).

METHODS

General

Experiments were conducted using male Sprague-Dawley rats weighing between 250 and 350 g. The animals were anesthetized with i.p. injections of pentobarbital (65 mg/kg body weight) for all procedures. Body temperature in anesthetized rats was maintained at 37–37.5°C using a rectal thermometer and a feedback-controlled heating pad. Cannulas (PE50) were placed in the femoral vein for the infusion of solutions and the injection of radioisotope, and in the femoral artery for the measurement of blood pressure and blood constituents.

Three groups of experiments were performed. In the first group, the effect of elevating the concentration of plasma taurine on the influx rate constant (K_1) for [^{3}H]taurine was measured. In the second group, [^{3}H]taurine influx rate constants were determined during osmotic stress. Finally, the effects of 4 h of MCAo were examined in a third group of rats. These animals were either used to measure brain taurine uptake, the uptake of [^{3}H]α-aminoisobutyric acid (AIB), a passive permeability marker (Blasberg et al., 1983), brain taurine content, or tissue water content. The AIB experiments were included to examine whether the measured changes in taurine uptake were due to an alteration in BBB passive permeability, whereas the measurement of taurine content enabled an assessment of the taurine efflux and the importance of changes in taurine influx in the overall changes in brain taurine. Water content measurements were used to account for the effects of tissue swelling on these parameters.

Taurine infusion experiments

Animals received artificial plasma with or without 50 m*M* taurine at a volume of 10 μl/100 g/min i.v. for 35 min. After 30 min, an arterial blood sample was taken for the measurement of blood gases, osmolality, electrolytes, and plasma taurine. Then the taurine influx rate constant was determined with [^{3}H]taurine, using [^{14}C]inulin as a plasma volume marker.

Osmotic stress experiments

Animals were divided into three groups. Hypo- or hyperosmotic stress was induced by i.p. injections of 10 ml/100 g distilled water or 2 ml/100 g 1.5 *M* NaCl, respectively. Controls received 2 ml/100 g 0.9% NaCl. After 30 min, an arterial blood sample was taken for measurements of blood gases, plasma osmolality, and electrolyte and taurine concentrations before administration of [^{3}H]taurine and [^{14}C]inulin for the determination of taurine uptake.

MCAo experiments

MCAo was induced by the intraluminal thread occlusion method as previously described (Longa et al., 1990) with minor modifications. Briefly, a midline incision was made in the ventral neck region for dissection of the common carotid artery bifurcation, taking care not to injure the adjacent vagal nerve. After cauterization of the pterygopalatine artery and branches of the external carotid artery, a 3–0 monofilament thread, rounded at the tip with a high-temperature ophthalmologic cautery device, was introduced retrogradely into the external carotid artery and advanced cranially in the internal carotid artery over a distance of exactly 19 mm, as measured from the bifurcation of the common carotid artery. The skin was then sutured and the animals were kept warm while recovering from anesthesia.

After 3.5 h, the animals were reanesthetized and cannulas were placed in the femoral artery for blood sampling and for the measurement of blood pressure. In those animals receiving radioisotope, an additional cannula was placed in the femoral vein. The rats were either used for the determination of cerebral tissue water content, tissue taurine content, [^{3}H]taurine influx, or [^{3}H]-AIB influx. Experiments were terminated after 4 h by decapitation followed by rapid removal of the brains. In all animals, ischemic cortex was discernible as well-demarcated, pale tissue surrounding the MCA. The cortical mantles of both hemispheres were rapidly dissected from underlying structures and flattened on a piece of Parafilm. A cork borer with a diameter of 5 mm was used to punch a tissue sample from the center of the MCA distribution cranially to the rhinal fissure (Fig. 1) of both hemispheres. Samples were immediately weighed and appropriately treated for the determination of tissue water content, taurine content, or the influx rate constants of [^{3}H]taurine and [^{3}H]-AIB.

Taurine influx rate constant and BBB passive permeability

The permeability of the BBB to [^{3}H]taurine and [^{3}H]-AIB was assessed by a modification of the method proposed by Ohno et al. (1978) for a single time-point analysis as previously described (Betz and Coester, 1990). For measurement of taurine uptake, a bolus of 0.2 ml saline containing 15 μCi [^{3}H]taurine was administered 5 min before decapitation of rats. In other animals of the MCAo group, 15 μCi [^{3}H]-AIB in 0.2 ml saline was allowed to circulate for 10 min before being killed for the determination of BBB passive permeability. After radioisotope injection, an arterial blood sample was continu-

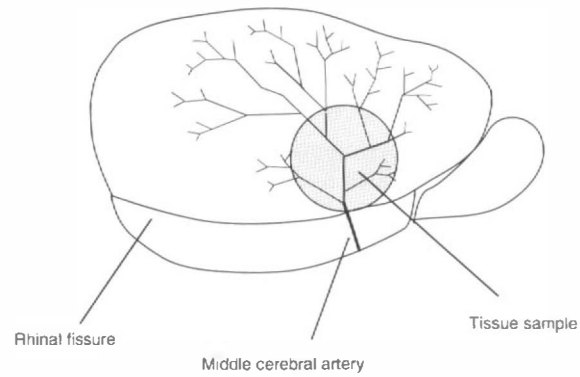

FIG. 1. Schematic representation of an occluded middle cerebral artery in a rat and location of the tissue sample in which the influx rate constants of taurine and [^{3}H]α-aminoisobutyric acid, and the tissue contents of taurine and water were determined.

ously withdrawn at a constant rate to determine the integral of plasma radioactivity. For the simultaneous determination of plasma volume, an additional 0.2 ml bolus of saline containing 10 μCi [^{14}C]inulin was given 3 min before decapitation. At the end of the experiment, a CSF sample was taken from the cisterna magna and a terminal plasma sample was obtained. Then the animal was decapitated and the brain was removed. Tissue samples were taken from the frontal cortex in all experiments except those with MCAo, where the cortex was sampled as described previously herein.

Tissue samples were placed in a preweighed vial and weighed, then dissolved in 330 μl of 1 *M* methylbenzethonium hydroxide at 60°C before addition of 6.5 ml of scintillation fluid. Whole blood samples were similarly incubated (20 min), then bleached with 50 μl of 30% H_2O_2 before addition of scintillation fluid, whereas plasma samples were immediately diluted with methylbenzethonium hydroxide and scintillation fluid. CSF samples were treated as tissue samples. Dual label counting was performed using a Beckman LS 3801 liquid scintillation spectrometer (Fullerton, CA, U.S.A.).

The permeabilities of [^{3}H]taurine and [^{3}H]-AIB at the BBB were assessed using a two-compartment model for blood to brain transfer (Ohno et al., 1978). This model assumes that tracer entry into brain is proportional to the plasma concentration of the tracer, that there is no appreciable backflux within the period of tracer circulation, and that isotope exchange between CSF and brain extracellular space is negligible. These conditions are accomplished for the present experiments by sampling only cortex and by the shortness of tracer circulation (Smith and Rapoport, 1986). Therefore, a rate constant for unidirectional uptake (K_1) relating the brain concentration of tracer over time to the arterial plasma concentration can be defined as:

$$K_1 = C_{ev}(T)/\int C_a dt \quad (1)$$

where $C_{ev}(T)$ is the terminal (time = T) concentration of extravascular tracer in either the brain or CSF and $\int C_a dt$ is the integral of the arterial tracer concentration from time $t = 0$ to T. $C_{ev}(T)$ was calculated from total tracer counts $C_{tot}(T)$ in the brain samples, final tracer plasma concentration $C_{pl}(T)$, and plasma volume PV as:

$$C_{ev}(T) = C_{tot}(T) - [PV \times C_{pl}(T)] \quad (2)$$

A similar two-compartmental model was employed to determine a K_1 for uptake into CSF. With CSF it is impossible to sample the whole of the CSF system. However, care was taken that a similar volume of CSF (100 μl) be taken from each rat. Thus, approximately the same portion of the CSF was sampled in each animal, facilitating comparisons between groups. The K_1 refers to the entry into that sample. A plasma volume correction was not necessary for CSF samples. Unidirectional fluxes of taurine (J_{in}) from blood to brain and blood to CSF were calculated by multiplying the influx rate constant (K_1) by the plasma taurine concentration in each animal.

Tissue and plasma composition

Plasma [Na^+] and [K^+] were measured by flame photometry (IL 943 flame photometer, Instrumental Laboratories, Lexington, MA, U.S.A.), [Cl^-] by coulometry (Digital chloridometer, Haake-Buchler Instruments, Saddlebrook, NJ, U.S.A.), and osmolality by the vapor pressure method (Wescor 5100 C vapor pressure osmometer, Logan, UT, U.S.A.). Tissue water content (g H_2O/g dry weight) was determined from sample wet and dry weights after desiccation for 24 h at 100°C.

For the measurement of tissue taurine, weighed samples were homogenized in 2 ml of ice-cold 0.05 *M* $HClO_4$ at 20,000 rpm for 30 s (IKA T25-S1 homogenizer, Cincinnati, OH, U.S.A.) followed by centrifugation at 0°C and 5,000 *g* for 10 min. Supernatant (20 μl) was analyzed by HPLC using a 75 × 4.6-mm 3 μm Beckman Ultrasphere chromatography column heated to 45°C and an isocratic elution profile after precolumn derivatization with *o*-phthalaldehyde. Amino acids were detected electrochemically (model 400 EC detector, Princeton Applied Research). The mobile phase consisted of 80% 0.05 *M* NaH_2PO_4 at pH 5.5 and 20% methylalcohol (Murai et al., 1992). Plasma taurine was determined by the same method after deproteinization with methanol. Tissue taurine content was calculated relative to both tissue wet weight and dry weight. The latter value was obtained by dividing tissue taurine relative to tissue wet weight by tissue water content measured in separate animals.

Materials

Rats were purchased from Charles River Laboratories (Portage, MI, U.S.A.). Electrolytes were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.) and taurine and glucose were from Sigma (St. Louis, MO, U.S.A.). The [^{3}H]taurine and [^{3}H]-AIB were provided by New England Nuclear (Boston, MA, U.S.A.), [^{14}C]inulin by American Radiolabeled Chemicals (St. Louis, MO, U.S.A.), and methylbenzethonium hydroxide and Cytoscint scintillation fluid by ICN (Costa Mesa, CA, U.S.A.).

Statistical analysis

All data are reported as means ± standard deviation (SD). Statistical differences between ischemic and nonischemic cortices of the same animals were determined by the paired *t* test. Other statistical comparisons between the groups were by analysis of variance with posthoc Scheffé *F* test where necessary for comparisons of multiple groups. The rules of error propagation were applied for the calculation of tissue taurine contents relative to tissue dry weight from two independent experiments, and for the calculation of the ischemic taurine loss,

corrected for ischemic edema. Confidence intervals were calculated for these data to determine their statistical significance. Regression analysis was performed where appropriate. The α error probability for significant differences was set at $p < 0.05$ (two-tailed).

RESULTS

Taurine infusion

Physiological parameters measured in the taurine and vehicle infusion experiments are given in Table 1. Taurine infusion increased plasma taurine fourfold compared with vehicle-infused rats. All other physiological variables were not significantly different between the two groups.

In the anterior cortex, the influx rate constant (K_1) for [^{3}H]taurine was significantly reduced in taurine-infused compared with vehicle-infused rats (2.1 ± 0.5 and 7.3 ± 2.0 μl/g/min, respectively; $p < 0.001$). However, a similar comparison of the K_1 for entry into the CSF (taurine infusion, 0.6 ± 0.1 μl/g/min; vehicle infusion, 0.8 ± 0.3 μl/g/min) was not significant ($p > 0.18$). Figure 2A plots the relation between plasma taurine concentration and the K_1 for the anterior cortex and CSF. For the anterior cortex, K_1 decreased as plasma taurine increased, approaching the abscissa with high taurine levels. Such a relationship would be expected in the presence of saturable uptake with a low Michaelis-Menten constant (K_M). Conversely, no relationship was found at the blood–CSF barrier, where K_1 remained constant in the face of increasing plasma taurine, as would be expected of a diffusional process or a transporter with a high K_M.

Unidirectional taurine fluxes (J_{in}) were calculated for all animals from K_1 and plasma taurine measurements. The J_{in} for entry at the blood–brain barrier was not increased during taurine infusion, probably indicating carrier saturation (Fig. 2B). However, the J_{in} into CSF was not kept constant, being significantly higher in taurine-infused rats ($p < 0.001$).

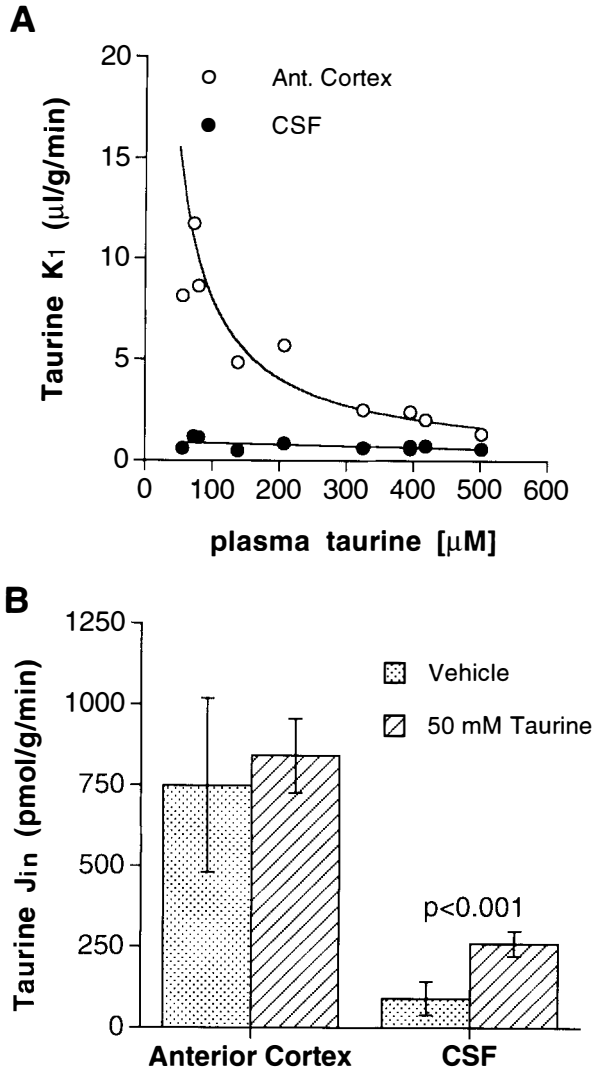

FIG. 2. A: Changes in taurine influx rate constant (K_1) in anterior cortex and CSF in response to varying plasma taurine concentrations obtained in vehicle- and taurine-infused rats. Saturable uptake was evident for anterior cortex where K_1 decreased with increasing plasma taurine concentration but not for CSF, where the K_1 remained unchanged. **B:** Taurine unidirectional influxes (J_{in}) in anterior cortex and CSF in vehicle- and taurine-infused rats. Values are means ± SD; n = 5.

Osmotic stress

Osmotic stress induced profound changes in the plasma taurine concentration (Table 1). Hypo-, iso-, and hyperosmotic groups, with osmolalities of 258 ± 7, 288 ± 7, and 346 ± 19 mOsmol/kg and corresponding disturbances in plasma Na^+ and Cl^-, had plasma taurine concentrations of 400 ± 186, 100 ± 33, and 47 ± 4 μ*M*, respectively. All animals in these groups developed slight metabolic acidosis.

The measurements of K_1 for [^{3}H]taurine obtained during osmotic stress had a similar pattern to that found with taurine infusion. In the anterior cortex, the taurine K_1 was reduced in hypoosmotic rats where plasma taurine was high and increased in response to hyperosmolality where plasma taurine was low (Fig. 3A). The J_{in} of taurine into brain, therefore, remained constant irrespective of osmolality (Fig. 3B). In contrast, the CSF K_1 was unaffected by changes in osmolality and, therefore, J_{in} was increased in hypoosmotic rats where plasma taurine concentrations were increased.

MCAo

Plasma taurine, 86 ± 31 μ*M*, after 4 h of MCAo, was similar to that of the control animals from the other two sets of experiments. Although the K_1 for taurine in the contralateral cortex (6.1 ± 0.9 μl/g/

TABLE 1. *Physiological parameters measured in rats after 30 min of taurine or control infusion, 30 min of osmotic stress, or 4 h of middle cerebral artery (MCA) occlusion*

Group	Taurine infusion	Control infusion	Hypoosmotic	Isoosmotic	Hyperosmotic	MCA occlusion
n	5	5	6	6	6	18
$[taurine]_{pl}$ (μM)	407 ± 63[a]	110 ± 62	400 ± 186[a]	100 ± 33	47 ± 4[b]	86 ± 31
$[K^+]_{pl}$ (mM)	3.7 ± 0.3	4.0 ± 0.3	4.3 ± 0.5	3.5 ± 0.3	3.4 ± 0.2[a]	4.0 ± 0.5
$[Na^+]_{pl}$ (mM)	138 ± 1	134 ± 3	125 ± 5[a]	145 ± 2	173 ± 8[a]	143 ± 4
$[Cl^-]_{pl}$ (mM)	109 ± 3	107 ± 2	90 ± 8[a]	106 ± 4	140 ± 12[a]	108 ± 4
Osmolality (mOsmol/kg)	286 ± 4	289 ± 4	258 ± 7[a]	288 ± 7	346 ± 19[a]	295 ± 6
pH (mm Hg)	7.37 ± 0.05	7.40 ± 0.03	7.34 ± 0.05	7.31 ± 0.05	7.26 ± 0.02[c]	7.38 ± 0.03
Po_2 (mm Hg)	79 ± 7	79 ± 10	93 ± 16	86 ± 13	98 ± 5	69 ± 10
Pco_2 (mm Hg)	58 ± 10	50 ± 6	50 ± 2	53 ± 7	50 ± 10	48 ± 4
Hematocrit (%)	42 ± 2	41 ± 2	49 ± 2[a]	40 ± 2	40 ± 2	42 ± 2
MABP (mm Hg)	N.D.	N.D.	111 ± 15	100 ± 8	107 ± 2	83 ± 8

Values are means ± SD. N.D., not determined. For the infusion experiments rats were given artificial plasma with or without 50 mM taurine at 10 μl/100 g/min i.v. for 35 min. Blood was sampled at 30 min, immediately before administration of isotope. For osmotic stress experiments, blood was sampled 30 min after the onset of the stress just before the determination of the influx rate constant. In the MCA occlusion group, data are pooled from the four different experimental groups (see text) because there were no significant differences between the groups except plasma taurine, which was only determined in those rats (n = 12) used for brain taurine uptake or content measurements. [a]$p < 0.01$ versus control, [b]$p < 0.05$ versus control, [c]$p < 0.05$ versus hypoosmotic rats.

min) was similar to that obtained in the other control animals, there was a very pronounced (82 ± 4%) reduction in the taurine K_1 to 1.1 ± 0.2 μl/g/min in the ischemic tissue (Fig. 4; $p < 0.001$).

Two possible causes for the reduction in the taurine K_1 were examined. The swelling of the ischemic tissue through edema formation will decrease the BBB surface area per unit wet weight. Four hours of ischemia resulted in the water content of the ischemic tissue increasing to 4.32 ± 0.16 g/g dry weight compared with 3.51 ± 0.05 g/g dry weight in the contralateral hemisphere (Fig. 5). This edema formation decreased the dry weight per wet weight of brain by 15%. Assuming that capillary surface area varies with the dry weight fraction, this difference would not account for the measured change in taurine K_1.

In other animals the possibility that the change in taurine K_1 with ischemia might reflect a change in the surface area or passive permeability properties of the BBB was examined by measuring the K_1 for AIB. Four hours after MCAo there was no increase in the AIB K_1, as might be expected if the BBB were disrupted; rather, the K_1 was decreased (Fig. 4; $p < 0.05$). The decrease in the K_1 for AIB, 41 ± 32%, was less than the 82 ± 4% reduction found for taurine ($p < 0.05$).

After 4 h, taurine content in the ischemic tissue was 4.8 ± 0.5 mmol/kg wet weight, significantly less than in the contralateral tissue (7.4 ± 0.8 mmol/kg; $p < 0.001$). Even if the tissue content is expressed per kilogram dry weight, there was significantly less taurine in the ischemic tissue (Fig. 5). The difference between ischemic and contralateral hemispheres was 7.4 ± 4.7 mmol/kg dry weight (95%

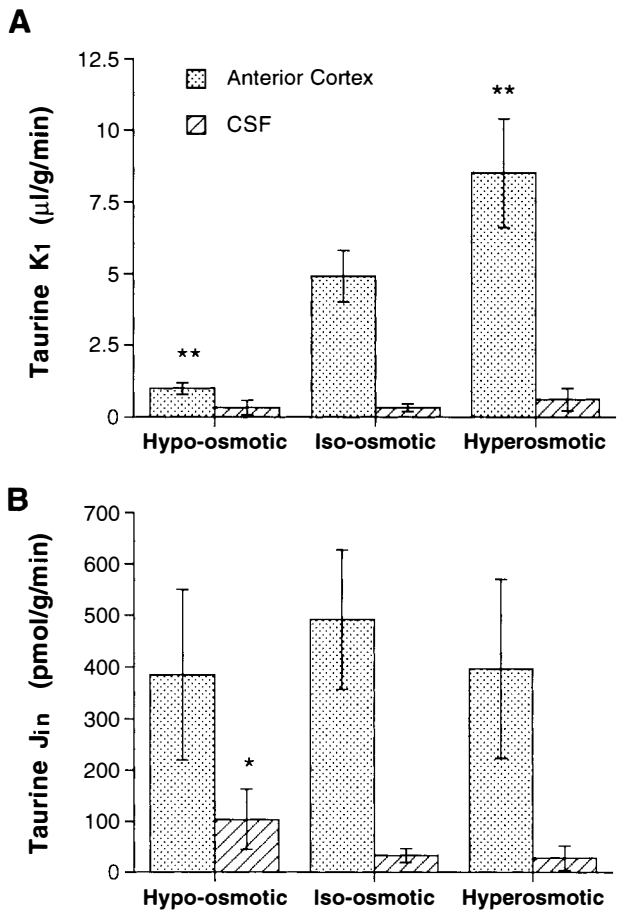

FIG. 3. Changes in **(A)** taurine influx rate constant (K_1) and **(B)** unidirectional taurine influx (J_{in}) in anterior cortex and CSF in response to osmotic stress. Values are means ± SD, n = 6. **$p < 0.001$ versus control; *$p < 0.01$ versus control and hyperosmotic groups.

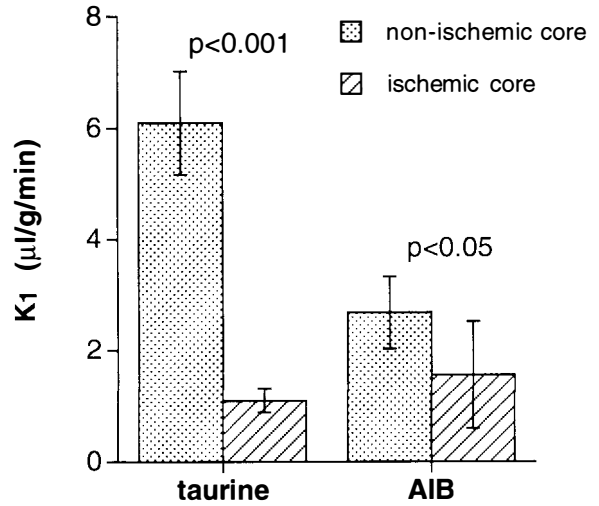

FIG. 4. Influx rate constants (K_1) for taurine and [^{3}H]α-aminoisobutyric acid (AIB) in the ischemic and nonischemic core samples after 4 h of middle cerebral artery occlusion. Values are means ± SD, n = 6.

lower confidence interval: 2.5 mmol/kg dry weight), corresponding to a 22% taurine loss. The rate of taurine loss was calculated as 31 ± 20 nmol/g dry weight/min or 6.8 ± 4.3 nmol/g wet weight/min (95% lower confidence interval: 2.3 nmol/g wet weight/min).

DISCUSSION

Taurine has been implicated in a variety of physiological and pathophysiological processes and, therefore, an understanding of the mechanisms that control brain taurine is important. Because appreciable taurine biosynthesis in the CNS seems to be lacking, brain tissue relies on taurine transported from blood across the BBB to meet its metabolic needs (Huxtable, 1989). The results presented in this article suggest that the influx of taurine into the brain is via a specific transporter that maintains influx constant during fluctuations in plasma taurine

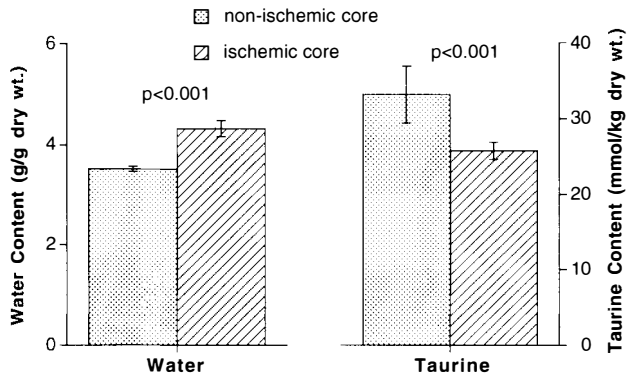

FIG. 5. Ischemic and nonischemic core water and taurine contents after 4 h of middle cerebral artery occlusion. Values are means ± SD, n = 6.

induced by taurine infusion or osmotic stress. However, during ischemia there may be a derangement of taurine influx.

Saturable uptake of taurine at the BBB

The present investigation provides evidence for saturable, i.e., carrier-mediated transfer of taurine from blood to brain across the BBB. While a diffusion component of taurine across the BBB cannot be ruled out, its contribution to taurine uptake at physiological plasma levels of the amino acid is presumably small compared with the transport component, as can be judged from the influx rate constants (K_1) measured at high plasma taurine levels. The data suggest that the K_M for taurine influx (J_{in}) at the BBB is substantially less than the normal taurine concentration found in rat plasma, and it may be a high-affinity uptake system as found in cultured neurons and astrocytes (Borg et al., 1980; Larsson et al.; 1986) and isolated capillaries (Tayarani et al., 1989).

In contradistinction to the BBB, the CSF K_1 remained unaffected by increasing plasma taurine concentrations, indicating the predominance of diffusion or a low-affinity transporter for taurine movement across the choroid plexus epithelium, the blood–CSF barrier. Conceivably, radioactivity accumulating in CSF during the experiment could have been the result of diffusion or bulk flow from tissue. However, the period of tracer recirculation was too short to allow significant exchange (Smith and Rapoport, 1986). Furthermore, if meaningful amounts of tracer had reached CSF from brain interstitial fluid, the K_1 calculated for CSF would have been expected to follow the pattern established for tissue, which was not the case.

The saturation of blood–brain barrier taurine transport may be important in guarding the brain from fluctuations in plasma taurine concentration. Lefauconnier et al. (1978) have shown that even very large increases in plasma taurine are not reflected in changes in brain taurine content. This observation is explained by the present data, because increases in plasma taurine would not be accompanied by increases in J_{in}.

Taurine transport during osmotic stress

From the results presented in this article, it is unlikely that changes in taurine unidirectional influx are involved in the net loss of brain taurine that occurs during acute hypoosmotic stress (Verbalis and Gullands, 1991; Sterns et al., 1993). Such loss probably reflects increased efflux through nonsaturated abluminal transport systems after an increase in the taurine concentration in the extracel-

lular space (see later in this article). However, the kinetics of blood to brain taurine transport do serve to maintain the J_{in} for taurine constant in the face of substantial changes in plasma taurine induced by osmotic stress. If the carrier were not saturated, the fourfold rise in plasma taurine that occurs during hypoosmotic stress would increase the rate of taurine uptake, thereby decreasing the net loss of taurine from the brain. Similarly, if the carrier were not saturated, the reduction in plasma taurine that occurs in hyperosmotic stress would diminish influx and thereby limit the net uptake of taurine that occurs in this condition to attenuate brain shrinkage (Trachtman et al., 1988; Thurston et al., 1983).

The absence of any effect of hypoosmotic stress on J_{in} suggests that brain swelling does not trigger regulators of this parameter. This makes it unlikely that the apparent decrease in taurine uptake found during ischemia is a regulatory event to limit edema formation.

Taurine transport during ischemia

In contrast to previous studies, which found little if any effect of ischemia or anoxia on the K_1 for another amino acid, leucine, at the BBB (Betz et al., 1975; Sage et al., 1984; Brust, 1991), cerebral ischemia induced a very marked decline in this parameter for taurine. This reduction, ~80%, could not be accounted for by tissue edema. Similarly, measurements of the K_1 for AIB suggest that the reduction in taurine K_1 cannot be accounted for by changes in the surface area of the capillary network because that would equally affect both AIB and taurine. They also suggest that any reduction in the passive permeability of the BBB is not of a magnitude that would reduce taurine influx by this extent. The BBB permeability to taurine is also so low that it is unlikely that the rate of taurine uptake would be limited by the rate of blood flow to the ischemic tissue even in the core, where blood flow is reduced to 10–15% of control (Yang and Betz, 1994).

One plausible explanation for the decrease in the K_1 of taurine is that the concentration of unlabeled taurine increases in blood as it circulates through the brain, competing with the isotopic taurine for uptake. The ischemic tissue lost taurine at the rate of 7 nmol/g wet weight/min. Assuming that this is all lost to blood flowing at a rate of 100 μl/g/min with a hematocrit of 40%, this would result in an increase from 86 to 203 μ*M* in the plasma taurine concentration during passage through the ischemic tissue. Because the taurine transporter appears saturated, such a doubling in concentration could greatly reduce the influx rate constant. As in osmotic stress, if this scenario occurs, the saturation of the taurine influx transporter serves to prevent taurine lost to the blood from being taken back up into the brain.

Another possible, and not mutually exclusive, cause for the decline in taurine K_1 may be an effect of ischemia on the endothelial Na gradient. Na gradients drive taurine influx in many tissues (Huxtable, 1989), including the abluminal transporter in cerebral microvessels where external Na removal or addition of ouabain inhibits taurine uptake (Tayarani et al., 1989). If MCAo alters the Na gradient of the cerebral endothelial cells, as occurs in other brain tissues, it would result in a decreased rate of taurine uptake. Interestingly, the uptake of leucine into the brain, which is via a sodium-independent L-system, is not inhibited by anoxia (Betz et al., 1975). If the decrease in taurine uptake does reflect a change in endothelial cell Na gradient, this change precedes BBB disruption. Thus, potentially, BBB taurine transport could be used as an early marker for BBB dysfunction.

Role of taurine in cerebral ischemia

During 4 h of MCAo there is a net increase in the tissue contents of the major ions Na^+, K^+, and Cl^- of ~60 mmol/kg dry weight (unpublished data). The loss of taurine measured in this study, amounting to ~7 mmol/kg dry weight, will therefore suffice to reduce this osmole gain by ~10% and may alleviate the evolution of tissue edema accompanying focal ischemia.

The loss of taurine could theoretically either result from enhanced taurine efflux or reduced influx, because appreciable metabolism of taurine in cerebral cells is lacking (Walz and Allen, 1987). Even with the almost total abolishment of taurine uptake, the reduction in influx only amounts to ~0.6 mmol/kg dry weight during 4 h. Thus, the major cause of the loss of taurine must be accelerated efflux. The increase in efflux was observed in the face of an intact BBB, as demonstrated by the passive permeability marker [^{3}H]-AIB, suggesting that the efflux is carrier mediated. The observed increase in efflux, ~7 mmol/kg dry weight/4 h is much greater than the capacity for blood to brain taurine transport. This observation is in accord with the finding of low brain extracellular space taurine concentrations, as measured at the CSF, of only 10 μ*M* (Semba and Patsalos, 1993) compared to 100 μ*M* in plasma. Tayarani et al. (1989) have estimated that the K_M for Na-dependent taurine transport from brain to blood is ~30 μ*M* and, thus, unlike the blood to brain transport, this system is probably not saturated under normal physiological conditions. Such an efflux system would be stimulated during ischemia, when extracellular taurine concentration increases

(Butcher et al., 1990; Shimada et al., 1993; Korf et al., 1988).

If, as suggested previously herein, the reduction in taurine influx during ischemia represents a reduction in the endothelial Na gradient that is linked to taurine transport, the dissipation of this gradient will also affect taurine efflux from the brain. Endothelial cell dysfunction, therefore, may limit taurine extrusion from the brain and lessen a mechanism that would normally limit brain swelling.

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