Amino Acids (2004) 27: 261–268
DOI 10.1007/s00726-004-0139-8

Amino Acids
Printed in Austria

Characteristics of basal taurine release in the rat striatum measured by microdialysis

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Received June 2, 2004
Accepted August 31, 2004
Published online November 12, 2004;

Summary. Taurine is a sulfur-containing amino acid thought to be an osmoregulator, neurotransmitter or neuromodulator in the brain. Our objective was to establish how much taurine is released in the striatum and examine the mechanisms controlling extracellular taurine concentrations under resting conditions. The experiments were made on rats by microdialysis *in vivo*. Changes in taurine were compared with those in glutamate, glycine and the non-neuroactive amino acid threonine. Using the zero net flux approach we showed the extracellular concentration of taurine to be 25.2 ± 5.1 μM. Glutamate was increased by tetrodotoxin and decreased by Ca^{2+} omission, glycine and threonine were not affected and both treatments increased extracellular taurine. The basal taurine release was increased by the taurine transport inhibitor guanidinoethanesulfonate and reduced by the anion channel blocker 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid.

Keywords: Taurine – Glutamate – Glycine – Threonine – Zero net flux – Synaptic transmission

Introduction

Taurine (2-aminoethanesulphonic acid) is a non-essential amino acid abundant in the central nervous system (CNS) of mammals. It is released from nervous tissue by hypoosmotic stimuli and regulates cell volumes (Oja and Saransaari, 1996; Hussy et al., 2000; Pasantes-Morales et al., 2002; Kreisman and Olson, 2003). However, taurine is highly heterogeneously distributed in the brain (Kontro et al., 1980; Palkovits et al., 1986; Pow et al., 2002). It may therefore serve other functions aside from osmoregulation, being specific for different brain regions. Taurine has been thought to be a neuromodulator or transmitter in the brain (Oja and Kontro, 1983). Indeed, many characteristics of taurine release, uptake and neuronal effect correspond well to those of neurotransmitters. Application of taurine generally modifies neuronal electrical potentials by increasing Cl^- conductance and evokes hyperpolarization (Oja et al., 1990; Belluzzi et al., 2004). In the striatum it also potentiates synaptic transmission (Chepkova et al., 2002). Taurine is released from nervous tissue in response to depolarizing agents such as N-methyl-D-aspartate (NMDA) and high concentrations of K^+ (Kontro and Oja, 1987d; Menendez et al., 1993; Semba et al., 1995; Saransaari and Oja, 1991, 1997, 2003), and sequestered by active high-affinity uptake systems (Kontro and Oja, 1978; Oja and Kontro, 1984). Extracellular taurine modifies the release of amino acid transmitters and modulates intracellular Ca^{2+} homeostasis (Kamisaki et al., 1993; Foos and Wu, 2002).

Many specific questions, however, remain open, hampering clear conclusions as to the neurotransmitter or neuromodulatory functions of taurine. In first place, the target of taurine action in the brain has not been defined. Many neuronal effects of taurine are diminished by GABA and glycine receptor antagonists (Malminen and Kontro, 1986; Kontro and Oja, 1987c; Ye et al., 1997; Chepkova et al., 2002; Belluzzi et al., 2004). A specific taurine receptor in the synaptic membranes has been described (Kontro and Oja, 1983, 1987a, 1987b; Frosini et al., 2003), but some investigators have failed to demonstrate it (Lähdesmäki et al., 1977; Lopez-Colomé and Pasantes-Morales, 1981). The origin of taurine release is also not precisely known. Many studies have failed to witness the neuronal origin of the K^+- or NMDA-evoked taurine release (Hanretta and Lombardini, 1987; Semba et al.,

1995). Taurine release is invariably slower in onset and offset (Korpi et al., 1981; Kontro and Oja, 1987d). The data concerning the accumulation of taurine in synaptic vesicles are somewhat contradictory (Kontro et al., 1980; Fyske and Fonnum, 1996). The conclusion must be that the mechanisms of taurine release are not necessarily the same as those of classical neurotransmitters.

One approach to make this situation clearer is to describe taurine release under resting conditions and to compare the data to the characteristics of neurotransmitter release. The aim of our study was thus to assess the order of magnitude of basal taurine release in the rat striatum *in vivo* and to characterize the regulating mechanisms. The results obtained were compared with the characteristics of glutamate release. The zero net flux approach was adopted to determine the extracellular taurine concentration. We also studied whether basal taurine release is regulated by the taurine uptake systems and which mechanisms are involved in maintaining basal release. The taurine transport inhibitor guanidinoethanesulfonate (GES) was used to evaluate whether taurine uptake is active. To ascertain the synaptic origin of taurine release, we tested how the release is affected by tetrodotoxin (TTX) and Ca^{2+} omission. Changes in the taurine concentration were compared with those of the neurotransmitter glutamate and the non-neuroactive amino acid threonine. In order to determine whether or not glycine acts as a neurotransmitter in the striatum, changes in its concentrations were also monitored. Since taurine release may greatly contribute to cell volume regulation, we also tested how the basal extracellular taurine level is affected by application of the anion channel blocker 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS).

Materials and methods

Animals

The studies were carried out on anesthetized adult Sprague-Dawley male rats (250–300 g weight). The experimental procedures were in accordance with the European Community Directive for the ethical use of experimental animals. All efforts were made to minimize both the suffering and the number of animals used.

Microdialysis surgery

Implantation of microdialysis probes was done on the day of the experiment under halothane narcosis. The animals were first anesthetized with 4% halothane in air and placed in a stereotaxic frame (David Kopf Instruments, Düsseldorf, Germany). The concentration of halothane was then reduced to 0.8–1.2% and body temperature kept at 37.5°C with a heating pad. Microdialysis probes of concentric design (4 mm length, 0.5 mm o.d.) were implanted in the striatum. The stereotaxic coordinates were as follows: AP +0.5, ML ±3.0, DV −6.5 relative to the bregma and dural surface, according to the atlas of Paxinos and Watson (1996). Probes were perfused with a CMA-102 pump (CMA/Microdialysis AB, Stockholm, Sweden) at a rate of 2 μl/min with artificial cerebrospinal fluid (aCSF) containing (mM): 120 NaCl, 2.8 KCl, 25 $NaHCO_3$, 1.2 $CaCl_2$, and 1.0 $MgCl_2$, at pH 7.2, adjusted by bubbling of the solution with 5% CO_2. Sampling commenced at least 90 minutes after probe implantation and the dialysate samples stored at −20°C until analyzed.

HPLC detection of amino acids

The concentrations of taurine, glutamate, threonine and glycine were measured using HPLC with fluorescent detection after precolumn derivatization with *o*-phtaldialdehyde (OPA) (Kendrick et al., 1996) using the system of Shimadzu Scientific Instruments (Kyoto, Japan). Derivatization was performed in a SIL-10AD autoinjector with the OPA reagent (OPA, 0.4 g/l, 0.25% mercaptopropionic acid and 2% methanol in 0.29 M borate buffer, pH 10.4). The OPA reagent (26 μl) was added to the sample (56 μl), mixed, incubated for 2 min at 4°C, and then injected into the column. The amino acid derivatives were separated using a C18-HC column, ODS 2.5 μm packing, 4.6 × 250 mm (Waters, UK) equipped with a precolumn (4 × 6 mm). The flow rate was set at 0.8 ml per minute. The low-pressure gradient mode was used for elution. The mobile phases were 10% methanol and 2% tetrahydrofuran in 0.067 M phosphate buffer, pH 6.8 (A), and 60% methanol and 2% tetrahydrofuran in water (B). The concentration of B during separation was gradually increased from 3% to 100% and then dropped to zero. Fluorescence was measured with an RF-10A detector using excitation and emission wavelengths set at 340 nm and 450 nm, respectively. The concentrations of amino acids in dialysates were calculated using external and internal standards with VPclass5 software.

Experimental design

Zero net flux experiments and basal taurine release. To ascertain the actual extracellular concentration of taurine we applied zero net flux approach (Lönnroth et al., 1987). At the beginning of the experiment three 20-min basal samples were collected. Thereafter, the probes were perfused with aCSF containing randomly 1, 5, 10, 30 and 50 μM taurine. Three samples were collected from each perfusate solution. The administration of the next solution started 20 min after the previous one.

Assays of TTX, calcium, GES and SITS dependency. In these experiments the animals were divided into five groups: control, TTX, Ca^{2+}-free, GES and SITS groups. The control group received no treatment and ten 20-min samples were collected during the course of the experiment. In the TTX group, three basal samples were collected at the beginning of the experiment, followed by perfusion of 10 μM TTX dissolved in aCSF. In the Ca^{2+}-free group, the probes were perfused with Ca^{2+}-free aCSF after basal sample collection (concentrations of other components in aCSF were kept unaltered). The SITS and GES groups were infused with either the anion channel blocker SITS (2 mM in aCSF) or the competitive inhibitor of taurine transport GES (1 mM in aCSF) via the microdialysis probes from the fourth sample onwards.

Data analysis

Zero net flux experiments and basal taurine release. The basal level of taurine in the dialysate was calculated as an average ± S.E.M. from the first three samples collected in each experiment after perfusion with aCSF. The actual concentration and extraction fraction of taurine were then obtained using linear regression analysis from the data at different taurine concentrations in the perfusion fluid (Harris 2003). The difference between the taurine concentration in the perfusate and the dialysate versus the taurine concentration in the perfusate was plotted. Thus, y and x-axes show the gain or loss of taurine during microdialysis and the concentration of taurine perfused, respectively. The slope of the straight line, calculated with Excel software, represents the extraction fraction. The intercept on

the *x*-axis represents the point where there is no net flux of taurine across the dialysis membrane. At this point, the concentration of taurine, added to the perfusion medium, is equal to the actual extracellular concentration of taurine.

TTX, calcium, GES and SITS dependency. The mean concentration of amino acids in the first three samples was considered as 100%. The changes in amino acid concentrations evoked by treatments are shown as percentages of this baseline value. The data obtained were compared with the corresponding control values from separate experiments. The statistical significance of differences was estimated using the Student's *t*-test. The level of significance was set to 0.05.

Results

Basal taurine release and actual extracellular taurine concentration

The results from the zero net flux experiments at different taurine concentrations showed good linearity (Fig. 1). The actual extracellular concentration of taurine in the rat striatum was estimated to be $25.2 \pm 5.1\,\mu$M (Table 1). The basal taurine release into the dialysates was $2.70 \pm 0.28\,\mu$M and the extraction fraction (*in vivo* recovery) was 0.079.

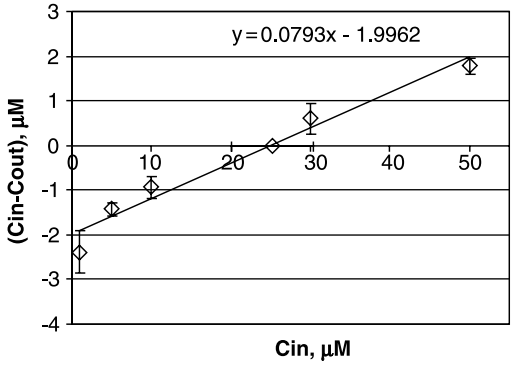

Fig. 1. Zero net flux experiments. The difference between the taurine concentrations in perfusate (C_{in}) and dialysate (C_{out}) is plotted against the concentration of taurine in perfusate. Number of experiments 12. Data are presented as mean values ± S.E.M.

Table 1. Estimated parameters of zero net flux experiment

Parameter	Value
Basal taurine release (perfusate concentration)	$2.70 \pm 0.28\,\mu$M
Slope (*in vivo* recovery, extraction fraction)	0.079
r^2 value of linear regression	0.963
Intercept with *x*-axis (extracellular concentration of taurine)	$25.2 \pm 5.1\,\mu$M

The data were estimated from 12 independent experiments. Mean values ± S.E.M.

Regulation of basal taurine release

The basal taurine release in these experiments ($2.65 \pm 0.20\,\mu$M, n = 22) did not differ significantly from the value obtained with the zero net flux experiments. During the course of sampling the release of taurine gradually decreased. The basal release of glutamate, threonine and glycine was 0.24 ± 0.03, 2.31 ± 0.15 and $1.31 \pm 0.08\,\mu$M, respectively (n = 22). The release of threonine and glycine was stable during the whole time course of the study, whereas the level of glutamate decreased.

Long-term application of the anion channel blocker SITS (2 mM, 140 min) significantly reduced the interstitial taurine concentrations (Fig. 2A). The maximal effect was discernible one hour after commencement of SITS perfusion, constituting a $39.1 \pm 4.6\%$ decrease. The

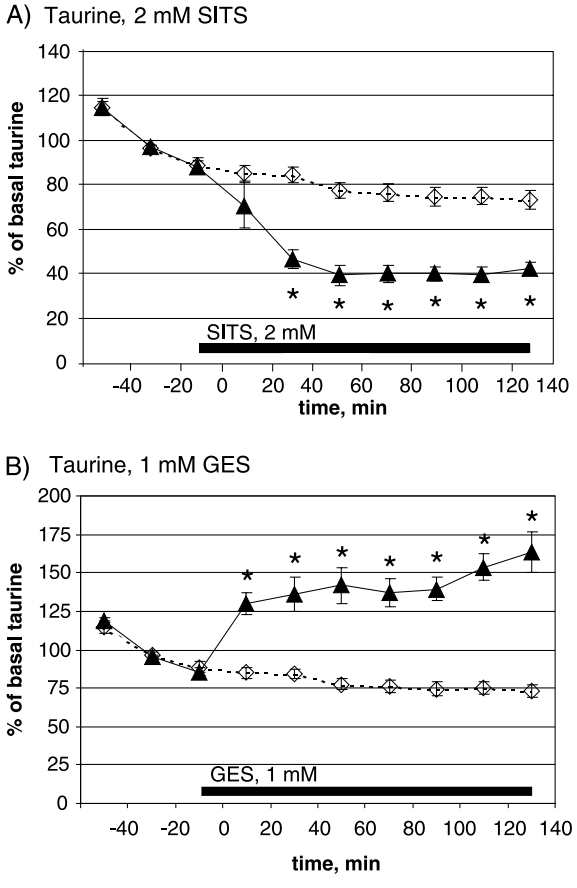

Fig. 2. Extracellular taurine concentrations under perfusion with 2 mM SITS (**A**) and 1 mM GES (**B**) (solid lines, n = 4). $^*P < 0.05$, compared to control values (dashed lines, n = 6). The solid bar denotes the period of SITS (**A**) or GES (**B**) perfusion. Data are presented as percentages ± S.E.M. from the baseline

Table 2. Effect of perfusion with 2 mM SITS and 1 mM GES on the extracellular concentrations of glutamate, glycine and threonine

Amino acid	Control % of the baseline	2 mM SITS % of baseline	1 mM GES % of baseline
Glutamate	82.8 ± 1.6	not estimated	80.7 ± 1.8
Glycine	101.6 ± 1.6	98.2 ± 1.8	97.2 ± 5.0
Threonine	105.9 ± 1.9	95.1 ± 2.1	101.7 ± 2.0

Data from 4–6 independent experiments, representing the mean values ± S.E.M. of the fourth to tenth samples (from 20 to 140 min). The baseline is the mean values of the three first samples before application of the effectors. In control experiments the rat brain was perfused with plain aCSF

concentrations of threonine and glycine were not affected by SITS (Table 2). Unfortunately, detection of glutamate was not possible due to the high degree of contamination by SITS at the beginning of the chromatograms.

The extracellular concentration of taurine was elevated by perfusion of the competitive taurine transport inhibitor GES (1 mM, 140 min; Fig. 2B). Taurine release was already significantly enhanced in the first sample after GES application. The maximal release (163.2 ± 13.0) was detected after 140 min. At the same time glutamate, threonine and glycine were not affected (Table 2).

Application of TTX (10 μM, 140 min) led to a gradual increase in the interstitial taurine concentration in the striatum of anesthetized rats (maximal increase was 110.0 ± 3.4% from baseline). The effect became statistically significant after 40 minutes of TTX perfusion (Fig. 3A). Glutamate was also released in response to TTX. The changes in glutamate were of the same magnitude as those in taurine (Fig. 3B). Perfusion with TTX did not influence the interstitial concentrations of threonine and glycine (Fig. 3C, D).

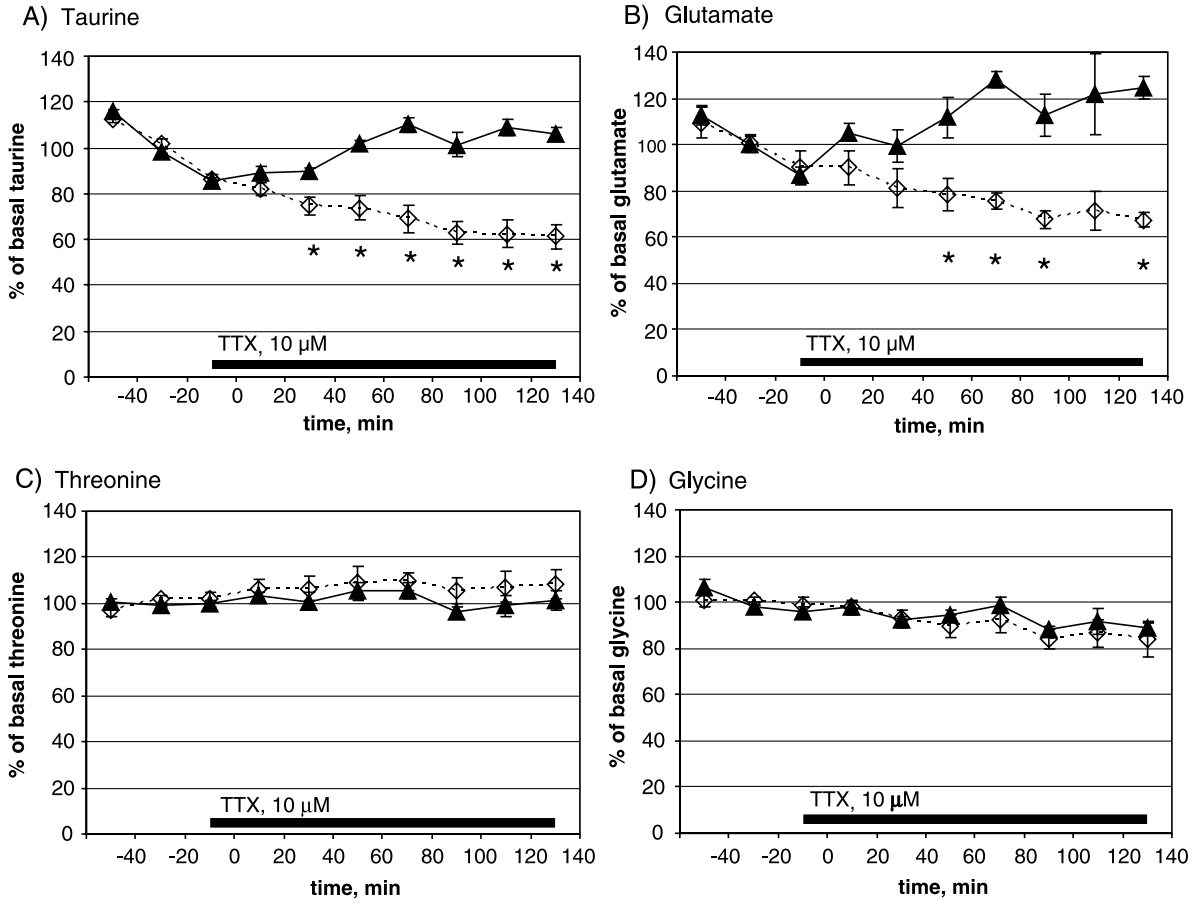

Fig. 3. Effect of perfusion with 10 μM TTX on the taurine (**A**), glutamate (**B**), threonine (**C**) and glycine (**D**) concentrations (solid lines, n = 4). $^{*}P < 0.05$, compared to control values (dashed lines, n = 4). The solid bar denotes the period of TTX perfusion. Data are presented as percentages ± S.E.M. from the baseline

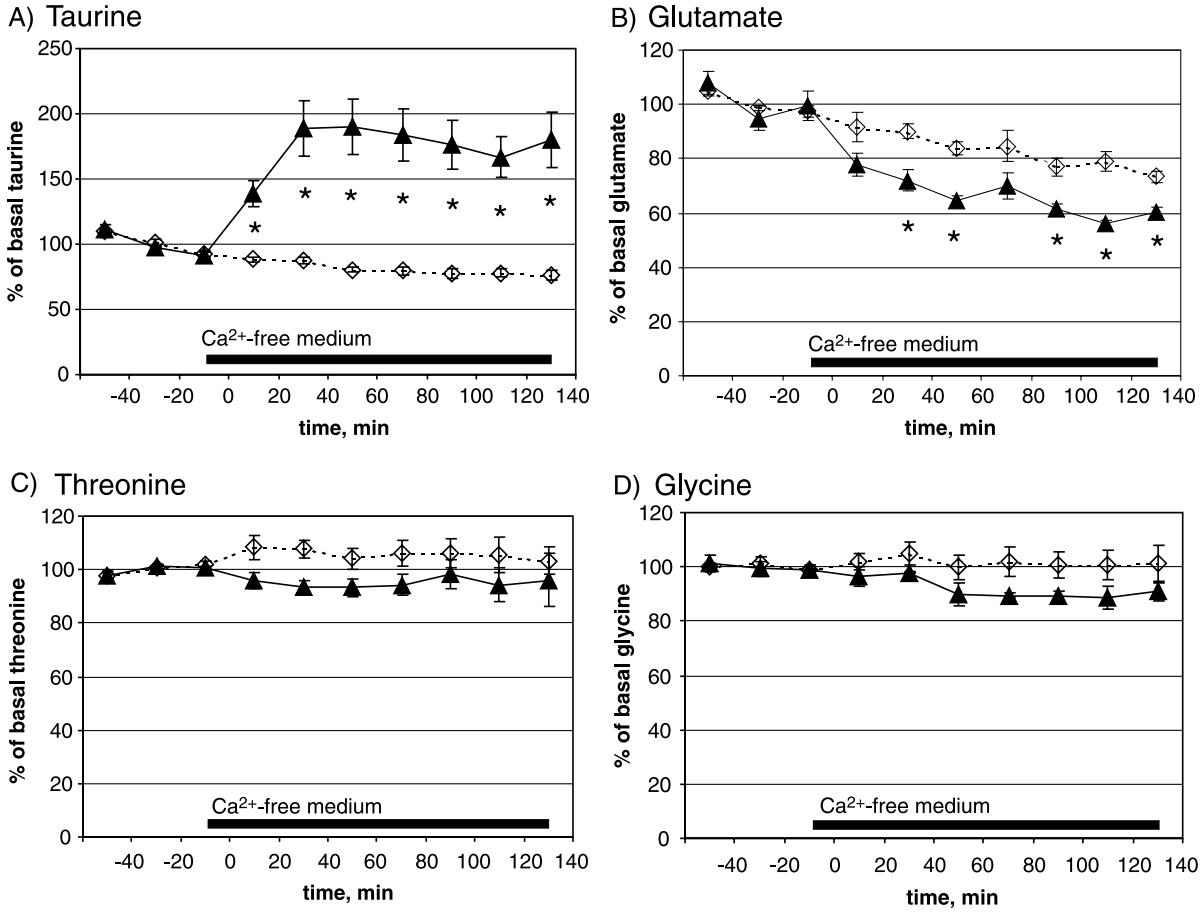

Fig. 4. Effect of perfusion with Ca^{2+}-free medium on the taurine (**A**), glutamate (**B**), threonine (**C**) and glycine (**D**) concentrations (solid lines, n = 4). $^{*}P < 0.05$, compared to control values (dashed lines, n = 6). The solid bar denotes the period of perfusion with Ca^{2+}-free medium. Data are presented as percentages ± S.E.M. from the baseline

Perfusion with Ca^{2+}-free aCSF during 140 min evoked a profound and stable increase in the taurine concentration in the dialysate, that was maximal after one hour (189.6 ± 21.2% from baseline, Fig. 4A). The effect was already statistically significant after 20 min of Ca^{2+}-free aCSF administration. Omission of Ca^{2+} led to a small but statistically significant decrease in the interstitial glutamate concentration, while the concentrations of threonine and glycine were not affected (Fig. 4B–D).

Discussion

Taurine abounds in the brain of many mammalian species at concentrations comparable to those of glutamate (Oja and Kontro, 1983; Huxtable, 1989), but its extracellular concentration in the rat striatum was now shown to be more than ten times higher that of glutamate (Miele et al., 1996; Lai et al., 2000). The present extraction fraction of taurine (*in vivo* recovery) does not markedly differ from the *in vitro* extraction fraction previously estimated in our laboratory (Hilgier et al., 2003). The fraction characterizes the penetration rate of taurine through the microdialysis membrane.

Taurine release evoked by ischemia, (Phillis et al., 1997), high potassium concentrations (Saransaari and Oja, 1998) and hypo-osmotic stimuli (Pasantes-Morales et al., 1990; Estevez et al., 1999) is partially mediated by volume-sensitive anion channels. SITS was used to evaluate whether this pathway is also active under resting conditions. This substance is a potent anion channel blocker and a weak inhibitor of the glutamate transporter. One mM SITS does not change the extracellular glutamate concentration and acts only on anion channels (Tauskela et al., 2003). We infer from the decrease in the extracellular concentration of taurine after SITS

infusion that about one half of taurine exits through the volume-sensitive chloride channels.

The taurine transporter TauT mediates taurine influx into cells. It belongs to the family of sodium- and chloride-dependent neurotransmitter transporters (Tappaz, 2004). The uptake is driven by the membrane gradient of Na^+ and regulated by the Ca^{2+}-calmodulin, protein kinase C and cAMP/protein kinase A pathways. The uptake of taurine is well characterized *in vitro* (Huxtable, 1989). Using the taurine transport inhibitor GES we now also demonstrated active uptake of taurine under resting conditions *in vivo*.

Two classical criteria for neurotransmitter release are the opening of fast sodium channels and Ca^{2+} dependency. Since the extracellular concentrations of both glutamate and taurine were increased by TTX application, but that of threonine not, the present TTX effect seems to be a characteristic of neuroactive amino acids. However, previous results on TTX effects on taurine and glutamate release are discrepant. For instance, the basal release of taurine in the substantia nigra *in vivo* and from brain slices *in vitro* has been slightly reduced or unaffected by inhibition of voltage-gated Na^+ channels by TTX (Biggs et al., 1995; Bianchi et al., 1999). In several studies no changes or a minor decrease in the glutamate concentration have been seen in response to inhibition of the voltage-gated sodium channels (for review see Timmerman and Westerink, 1997). However, in the studies specifically conducted on the striatum, extracellular glutamate has increased (Keefe et al., 1993; Morari et al., 1996). This phenomenon may reflect TTX-mediated disinhibition of cortical glutamatergic terminals. The firing of cortical and thalamic motoneurons is controlled by the striato-thalamo-cortical loop (Parent and Hazrati, 1995a, b). Under resting conditions the striatal efferents are silent, which leaves thalamic neurons suppressed. The dopaminergic striatonigral projections, which are tonically active, balance the activity of the two branches of the loop. Intrastriatal application of TTX attenuates dopaminergic tone and leads to disinhibition and subsequent release of glutamate from the cortical inputs.

Taurine was now released upon TTX application in a similar manner and in the same order of magnitude as glutamate. Taurine is thus either released from the same site (cortical inputs) or glutamate induces taurine release from other sources. It has previously been reported that decortication reduces the striatal levels of glutamate, but leaves the taurine concentrations unchanged (Butcher and Hamberger, 1987). The TTX-induced taurine elevation is hence likely to occur secondarily after the glutamate release. Although taurine may be released by a non-synaptic mechanism, the release is strongly coupled to changes in neuronal activity.

Due to methodological problems it is difficult to prove the Ca^{2+} dependency of the release measured by microdialysis. The basal glutamate release has been found to be unchanged or only slightly decreased after omission of Ca^{2+} (for review see Timmerman and Westerink, 1997). Here, a minor but statistically significant decrease in extracellular glutamate was likewise discernible in Ca^{2+}-free medium. The concentration of threonine was stable, indicating that Ca^{2+} omission does not evoke changes in non-neuroactive amino acids. In contrast, taurine release was enhanced in the absence of Ca^{2+}. Enhanced taurine release in the presence of Ca^{2+} chelators has already been seen in brain slices (Korpi and Oja, 1984; Oja et al., 1985; Saransaari and Oja, 1992), though the mechanism of this enhancement is not known. Interestingly, the omission of Ca^{2+} has no effect on taurine release in the supraoptic nucleus of the hypothalamus, where taurine is localized mainly in glia (Decavel and Hatton, 1995; Hussy et al., 2000). Since the localization of taurine in the striatum is predominantly neuronal (Storm-Mathisen and Ottersen, 1986; Madsen et al., 1987; Della Corte et al., 1990), enhanced taurine release in Ca^{2+}-free solution may be a phenomenon specific for neurons, but not for glia.

The taurine actions in the striatum are partially mediated by strychnine-sensitive glycine receptors (Chepkova et al., 2002). Glycine is a major inhibitory neurotransmitter in the spinal cord and brain stem. Recent studies have demonstrated the expression of functional glycine receptors in the forebrain neurons, but their physiological role is unknown (Sergeeva and Haas, 2001). Since glycinergic transmission in the forebrain has not yet been shown, these glycine receptors may be the target of either glycine or taurine (Flint et al., 1998; Hussy et al., 2000). In the rat striatum, strychnine-sensitive glycine receptors are present on cholinergic interneurons (Darstein et al., 2000), and taurine, together with glycine, is their highly potent agonist (Sergeeva and Haas, 2001). In our study, neither TTX nor Ca^{2+} omission had any effect on the extracellular concentration of glycine, signifying that taurine is rather the endogenous agonist of striatal glycine receptors.

Conclusions

We report that under resting conditions extracellular taurine concentrations are high and undergo dynamic

regulation by volume-regulated Cl^- channels and taurine transporter. In contrast to glutamate, Ca^{2+} depletion enhances basal taurine release suggesting that synaptic exocytosis is not involved. From the similar alterations in taurine and glutamate after TTX application we conclude that taurine release is related to neural activity but by means of some non-synaptic mechanism. Since neuronal activation alters ion gradients across cell membranes, taurine is likely to be released via osmotically induced pathways.

Acknowledgments

The support of the Centre for International Mobility (CIMO) and the Medical Research Fund of Tampere University Hospital is gratefully acknowledged.

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