Amino Acids (2007) 32: 439–446
DOI 10.1007/s00726-006-0375-1
Printed in The Netherlands

Amino Acids

Taurine release in mouse brain stem slices under cell-damaging conditions

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Received April 10, 2006
Accepted May 16, 2006
Published online September 27, 2006;

Summary. Taurine has been thought to be essential for the development and survival of neural cells and to protect them under cell-damaging conditions. In the brain stem taurine regulates many vital functions, including cardiovascular control and arterial blood pressure. We have recently characterized the release of taurine in the adult and developing brain stem under normal conditions. Now we studied the properties of preloaded [^{3}H]taurine release under various cell-damaging conditions (hypoxia, hypoglycemia, ischemia, the presence of metabolic poisons and free radicals) in slices prepared from the mouse brain stem from developing (7-day-old) and young adult (3-month-old) mice, using a superfusion system. Taurine release was greatly enhanced under these cell-damaging conditions, the only exception being the presence of free radicals in both age groups. The ischemia-induced release was characterized to consist of both Ca^{2+}-dependent and -independent components. Moreover, the release was mediated by Na^+-, Cl^--dependent transporters operating outwards, particularly in the immature brain stem. Cl^- channel antagonists reduced the release at both ages, indicating that a part of the release occurs through ion channels, and protein kinase C appeared to be involved. The release was also modulated by cyclic GMP second messenger systems, since inhibitors of soluble guanylyl cyclase and phosphodiesterases suppressed ischemic taurine release. The inhibition of phospholipases also reduced taurine release at both ages. This ischemia-induced taurine release could constitute an important mechanism against excitotoxicity, protecting the brain stem under cell-damaging conditions.

Keywords: Taurine release – Cell-damaging conditions – Ischemia – Ion channel inhibitors – Second messengers – Ca^{2+}-dependency – Brain stem slices – Adult and developing mice

Abbreviations: 9-AC, 9-antracenecarboxylate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; ATPase, adenosine triphosphatase; DIDS, diisothiocyanostilbene-2,2′-disulphonate; DNP, 2,4-dinitrophenol; GABA, γ-aminobutyrate; GAT, GABA transporter; H-7, (+)-1-(5-isoquinolinesulphonyl)-2-methylpiperazine; Hepes, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; IBMX, 3-isobutyl-1-methylxanthine; ODQ, 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one; PDE, phosphodiesterase; PMA, 4β-phorbol 12-myristate 13-acetate; PKC, protein kinase C; RO 20-1724, 4-(3-butoxy-4-methoxyphenyl)-2-imidazolidone; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonate

Introduction

Taurine has been suggested to be involved in osmoregulation and cell volume adjustments in the central nervous system (Huxtable, 1992; Oja and Saransaari, 1996). In addition, it has been thought to function as an inhibitory regulator of neuronal activity (Oja and Kontro, 1983a; Saransaari and Oja, 1992) and have a special role in immature brain tissue (Oja and Kontro, 1983a; Kontro and Oja, 1987; Sturman, 1993). It is essential for the development and survival of neural cells (see Huxtable, 1992; Sturman, 1993). Taurine also protects neural cells from excitotoxicity induced by excitatory amino acids in the hippocampus (French et al., 1986), cerebellum (Trenkner, 1990) and neuronal cultures (Tang et al., 1996), prevents harmful metabolic sequences evoked by ischemia or hypoxia (Schurr et al., 1987), and alleviates symptoms in epilepsy (Oja and Kontro, 1983b). In the hippocampus various cell-damaging conditions induce substantial release of taurine in both adult and developing rodents, particularly under ischemic conditions (Saransaari and Oja, 1997a, 1998a, 1999), thus providing a protective mechanism against excitotoxicity (Saransaari and Oja, 2000a).

The brain stem is an important brain region involved in the regulation of multiple sensory and visceral processes, being besides the location of cardiovascular and respiratory centres. Taurine has been suggested to participate in the modulation of cardiovascular control in the ventrolateral medulla (Kubo et al., 1993; Wang et al., 2005) and in the neurogenic control of arterial blood pressure in the nucleus of the solitary tract (Meeley et al., 1989). There is also evidence that the released taurine in the locus

coeruleus is involved in conditioned fear (Kaehler et al., 2000). The release processes of taurine under both normal and ischemic conditions have been studied both in vivo and in vitro in higher brain areas, including the hippocampus and striatum (Saransaari and Oja, 1992, 1997a, 1998a, 1999a; Phillis and O'Regan, 2003; Molchanova et al., 2004a, b, 2005). We have recently characterized the release of taurine in mouse brain stem slices under normal conditions (Saransaari and Oja, 2006). Now we complete this series of investigations by studying the release mechanisms of preloaded [^{3}H]taurine under cell-damaging conditions in slices prepared from the brain stem from developing (7-day-old) and young adult (3-month-old) mice, using a superfusion system. In particular, the release evoked by ischemia is characterized more closely.

Materials and methods

Materials

Young adult (3-month-old) and 7-day-old NMRI mice of both sexes were used in the experiments. [^{3}H]Taurine (specific radioactivity 1.15 PBq/mol) was obtained from Amersham International, Bristol, UK. The ion channel inhibitors were from Sigma (St. Louis, MO), except for 9-antracenecarboxylic acid (9-AC), which was together with other special reagents from Tocris Bioscience (Northpoint, Avonmouth, UK). Other reagents and drugs were from common commercial sources.

Release experiments

Superficial slices 0.4 mm thick weighing 15–20 mg were manually prepared from the mouse brain stem with a tissue slicer of Stadie-Riggs type. The slices were immediately immersed in 5 ml of oxygenated medium and incubated with 0.01 mM [^{3}H]taurine (50 MBq/l) at 37 °C for 15 min under agitation. The standard medium contained (in mmol/l) NaCl 127, KCl 5, $CaCl_2$ 0.8, $MgSO_4$ 1.3, Na_2HPO_4 1.3, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (Hepes) 15, NaOH 11 and D-glucose 10 (pH 7.4). The slices were then transferred into 0.25 ml cups and superfused with the above medium (unless otherwise specified) at a rate of 0.25 ml/min for a total of 50 min in a system in which freely floating shaken slices were kept under a continuous flow of oxygen in order to preserve their viability (Kontro and Oja, 1987). The superfusion medium was pooled during the first 20 min and thereafter 2-min fractions (0.5 ml) were directly collected into small scintillation vials with a fraction collector. After the 50-min superfusion the slices were weighed, homogenized in ice-cold 5% (w/v) trichloracetic acid solution, and centrifuged, and the clear supernatants were used for scintillation counting. The effluent samples were subjected to the same analyses.

Superfusion conditions

Neural cell damage was induced by modified experimental conditions. In hypoglycemia, glucose was omitted from the medium and in hypoxia the medium was bubbled with N_2 gas for 1 h before the experiments and then during the whole experimental period. In ischemia the glucose-free medium was bubbled with N_2 gas (Taylor et al., 1955). Free radical production was achieved by exposure to hydrogen peroxide (0.01%) (Pelmar, 1955). In Na^+-free media NaCl was equimolarly substituted by choline chloride added at the beginning of superfusion. In Cl^- free media Cl^- was replaced by acetate. In Ca^{2+}-free media, Ca^{2+} (0.8 mM) was omitted and ethylenediaminetetra-acetate (2.0 mM) added at the beginning of the superfusion experiments.

Estimation of efflux rate constants

Desaturation curves of labeled taurine from the slices were plotted as a function of time on the basis of the radioactivities remaining in the slices after superfusion and recovered in the collected superfusate fractions (Kontro and Oja, 1987). The efflux rate constants of taurine for the time intervals of 20–30 min (k_1) and 32–40 min (k_2) were computed as negative slopes for the regression lines of the logarithm of radioactivity remaining in the slices vs. superfusion time. During the period of 30–32 min the new medium applied replaced the preceding medium in superfusion chambers. The k_1 constants represent the basal prestimulation release from all slices and the subsequent k_2 constants the spontaneous or K^+-stimulated release of taurine. The comparisons of these k_2 constants enable estimation the magnitude of K^+ stimulation under different experimental conditions. In addition to k_1 and k_2, a third constant k_3 was calculated for the time interval of 40–50 min. The k_3 constants calculated were very similar to the respective k_2 constants and thus not shown.

Statistical calculations

The presence of statistically significant differences between the sample means was detected by variance analysis. Comparison of individual means was made by Hartley's sequential method of testing.

Results

The basal unstimulated release of [^{3}H]taurine from mouse brain stem slices was rather steady during the whole experimental time both in adult and developing mice (Figs. 1B, 2B). Cell-damaging conditions generally potentiated the release, with the exception of the presence of free radicals (0.01% hydrogen peroxide) (Figs. 1B, 2B). Hypoxia, hypoglycemia and ischemia were very effective in adults (Fig. 1A) and in 7-day-old mice (Fig. 2A). The effect of 0.1 mM 2,4-dinitrophenol (DNP) was the strongest in both ages, however (Figs. 1B, 2B).

The ischemia-induced taurine release was further characterized. K^+ stimulation was able to enhance the release significantly ($p < 0.05$), about 1.2-fold in adults, while the stimulation was around 2.5-fold ($p < 0.01$) in the immature brain stem (Table 1). Omission of Ca^{2+} did not affect the basal release in the 7-day-old mice, but K^+ stimulation was significantly diminished (Fig. 3, upper panel). In adults, the basal taurine release was significantly ($p < 0.05$) enhanced but no K^+ stimulation was discernible in the absence of Ca^{2+} (Fig. 3, lower panel). γ-Aminobutyrate (GABA) and taurine (both 1.0 mM) enhanced the release in both age groups (Fig. 4). Na^+ omission from the superfusion medium potentiated the ischemic taurine release in developing, but not in adult mice (Fig. 4). When Na^+ was omitted the potentiating effects of GABA and taurine were no longer discernible. When Cl^- was omitted the release was considerably ($p < 0.01$) increased in both age groups (Fig. 4).

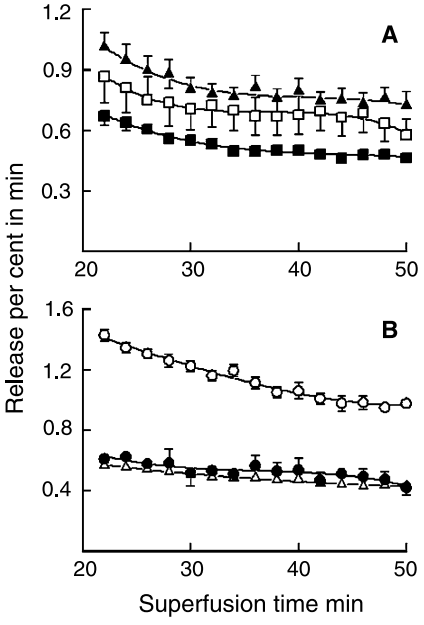

Fig. 1. Time-course of taurine release from brain stem slices from 3-month-old mice under cell-damaging conditions. Controls (-●-), the presence of 0.01% H_2O_2 (-△-) and 0.1 mM 2,4-dinitrophenol (-○-), hypoglycemia (-■-), hypoxia (-□-) and ischemia (-▲-). The slices were superfused in the above conditions as described in Materials and methods. The results in **A** and **B** are means of 4–8 independent experiments with SEMs indicated

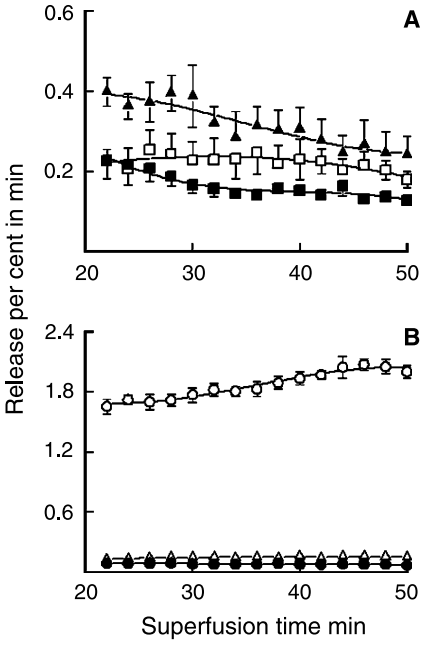

Fig. 2. Time-course of taurine release from brain stem slices from 7-day-old mice under cell-damaging conditions. Controls (-●-), the presence of 0.01% H_2O_2 (-△-) and 0.1 mM 2,4-dinitrophenol (-○-), hypoglycemia (-■-), hypoxia (-□-) and ischemia (-▲-). The slices were superfused in the above conditions as described in Materials and methods. The results in **A** and **B** are means of 4–8 independent experiments with SEMs indicated

The effects of various ion channel blockers were investigated by adding them to the superfusion medium under ischemic conditions at the beginning of the release experiments, and the subsequent K^+-stimulation was applied between 30 and 50 min (Table 1). The Cl^- channel blocker diisothiocyanostilbene-2,2′-disulphonate (DIDS, 0.5 mM) reduced the K^+-evoked release in both age groups and the initial basal prestimulated release (k_1 constant) in developing mice was also reduced. Another Cl^- channel blocker 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonate (SITS, 2.0 mM) reduced the basal and K^+-evoked release (k_2 constants) in both age groups. On the other

Table 1. Effects of ion channel inhibitors on taurine release from brain stem slices from 3-month- and 7-day-old mice in ischemia

Effectors	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM			
	3-Month-old		7-Day-old	
	k_1	k_2	k_1	k_2
Basal (control)	4.51 ± 0.21 (15)	3.72 ± 0.14 (11)	2.07 ± 0.20 (7)	1.31 ± 0.23 (8)
+ 50 mM K^+ (control)		4.55 ± 0.30 (8)		3.35 ± 0.25 (8)
DIDS 0.5 mM	4.07 ± 0.09 (9)	3.27 ± 0.13 (4)	0.89 ± 0.06** (7)	1.03 ± 0.13 (4)
+ 50 mM K^+		3.37 ± 0.21* (4)		1.93 ± 0.12** (4)
SITS 2.0 mM	3.00 ± 0.17** (8)	2.02 ± 0.15** (4)	0.90 ± 0.11** (8)	0.69 ± 0.06** (4)
+ 50 mM K^+		2.77 ± 0.19** (4)		1.75 ± 0.17** (4)
9-AC 0.2 mM	4.36 ± 0.19 (8)	4.54 ± 0.47 (8)	2.20 ± 0.14 (8)	1.92 ± 0.25 (4)
+ 50 mM K^+		5.67 ± 0.58** (7)		3.63 ± 0.27 (4)

The drugs were added at the beginning of superfusion and 50 mM K^+ at 30 min. The results show the efflux rate constants ± SEM ($\times 10^{-3}$ min^{-1}) for the time intervals of 20–30 min (k_1) and 32–40 min (k_2) with the number of independent experiments in parenthesis. Significance of differences from the corresponding controls: $^*p < 0.05$, $^{**}p < 0.01$

DIDS, diisothiocyanostilbene-2′2-disulphonate; *SITS*, 4-acetamido-4′-isothiocyanostilbene-2′2-disulphonate; *9-AC*, 9-anthracenecarboxylic acid

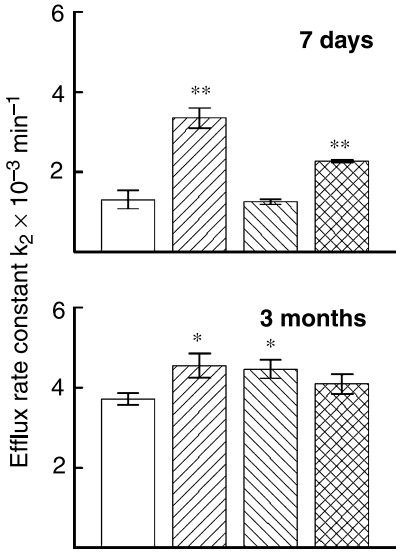

Fig. 3. Effect of Ca^{2+}-free medium on the efflux rate constants k_2 (32–40 min) of taurine release from brain stem slices from 7-day-old and 3-month-old mice in ischemia. The first open bars show the basal and the second right-hatched bars the K^+-stimulated release in Ca^{2+}-containing media, the third left-hatched bars the basal and the fourth cross-hatched bars the K^+-stimulated release in Ca^{2+}-free media. The results are mean values ± SEM of 4–8 independent experiments. Significance of differences from the basal release: $^*p < 0.05$, $^{**}p < 0.01$

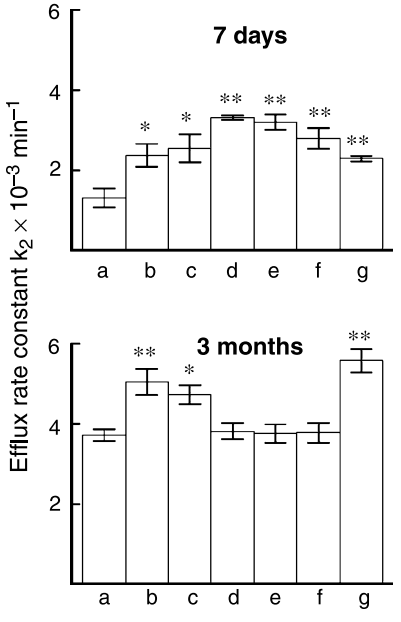

Fig. 4. Effects of Cl^- and Na^+ omission and structural analogues on taurine release from brain stem slices from 7-day-old and 3-month-old mice in ischemia. The bars show the efflux rate constants k_2 (32–40 min) as follows: (a) normal conditions (control), (b) 1.0 mM GABA added at 30 min in normal medium, (c) 1.0 mM taurine added at 30 min in normal medium, (d) Na^+-free medium, (e) 1.0 mM GABA added at 30 min in Na^+-free medium, (f) 1.0 mM taurine added at 30 min in Na^+-free medium, and (g) in Cl^--free medium. The results are mean values ± SEM of 4–8 independent experiments. Significance of differences from the controls: $^*p < 0.05$; $^{**}p < 0.01$

hand, the Cl^- transport inhibitor 9-anthracenecarboxylic acid (9-AC, 0.2 mM) had no effect on the release in either age group.

The effects of several compounds affecting the second messenger systems were investigated by superfusing the slices with these compounds from the beginning and during the subsequent K^+-stimulation up to 50 min under ischemia (Table 2). The tyrosine kinase inhibitor genistein (1 μM) had no effect on taurine release in immature mice, whereas the K^+-stimulated release in adults was significantly diminished. The phospholipase inhibitor quinacrine (0.1 mM) reduced the basal and K^+-evoked release in both age groups. Alloxan, an adenylyl cyclase inhibitor, had no effect on this ischemia-induced taurine release. The unspecific phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine (IBMX, 1.0 mM) and the selective PDE4 inhibitor 4-(3-butoxy-4-methoxyphenyl)-2-imidazolidone (RO 20-1724, 0.2 mM) both markedly diminished the basal and stimulated release in adult mice, but only the basal release in developing mice. Another PDE inhibitor zaprinast, selective for PDE5, 6 and 9, also inhibited the basal and K^+-evoked release at both ages, while the inhibitor of soluble guanylyl cyclase 1H-(1,2,4)oxadiazolo(4,3-a) quinoxalin-1-one (ODQ, 0.01 mM) reduced the basal release at both ages and also the K^+-evoked release in adults. Furthermore, the activator of protein kinase C 4β-phorbol 12-myristate 13-acetate (PMA, 10 nM) inhibited the initial basal release at both ages. Of the inhibitors of protein kinase C, chelerythrine (1 μM) had no effect on the release while (+)-1-(5-isoquinolinesulphonyl)-2-methylpiperazine (H-7, 0.1 mM) reduced both the basal and stimulated release in developing mice.

Discussion

The cell-damaging conditions applied generally potentiated taurine release in the brain stem, similarly to other brain regions (Saransaari and Oja, 1998a, b, 1999; Molchanova et al., 2004b), although the stimulatory effects have been larger in the hippocampus under the same conditions (Saransaari and Oja, 1997a). The only exception was the presence of free radicals, which now failed to have an effect. These conditions have induced a rather substantial release of taurine in the hippocampus (Saransaari and Oja, 1997a, 1998b, 2004). Apparently the brain stem is more resistant to that kind of damage than the hippocampus. Hypoxia, hypoglycemia, ischemia and free radical production cause neuronal cell damage and death. In ischemia, oxidative metabolism shifts to anaerobic glycolysis due to the lack of oxygen and glucose. The

Table 2. Effects of compounds involved in the second messenger systems on taurine release from brain stem slices from 3-month- and 7-day-old mice in ischemia

Effector	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM			
	3-Month-old		7-Day-old	
	k_1	k_2	k_1	k_2
Basal (control)	4.51 ± 0.21 (15)	3.72 ± 0.14 (11)	2.07 ± 0.20 (7)	1.31 ± 0.23 (8)
+ 50 mM K^+ (control)		4.55 ± 0.30 (8)		3.35 ± 0.25 (8)
Genistein, 0.001 mM	4.18 ± 0.17 (8)	2.95 ± 0.15** (4)	1.73 ± 0.21 (6)	1.34 ± 0.15 (4)
+ 50 mM K^+		3.78 ± 0.19 (4)		3.22 ± 0.32 (4)
Quinacrine, 0.1 mM	2.22 ± 0.18** (7)	1.76 ± 0.06** (4)	0.50 ± 0.03** (8)	0.50 ± 0.09** (4)
+ 50 mM K^+		1.90 ± 0.31** (4)		0.57 ± 0.02** (4)
Alloxan, 5.0 mM	4.30 ± 0.21 (7)	3.80 ± 0.13 (4)	1.92 ± 0.15 (8)	1.88 ± 0.21 (4)
+ 50 mM K^+		5.12 ± 0.13 (4)		3.10 ± 0.21 (4)
IBMX, 1.0 mM	3.50 ± 0.31* (8)	3.03 ± 0.29* (4)	1.37 ± 0.10** (8)	1.47 ± 0.27 (4)
+ 50 mM K^+		3.14 ± 0.20** (4)		2.33 ± 0.16* (4)
RO 20-1724, 0.2 mM	3.62 ± 0.15* (8)	3.08 ± 0.28** (8)	1.41 ± 0.09** (8)	1.22 ± 0.16 (4)
+ 50 mM K^+		3.55 ± 0.13* (4)		2.90 ± 0.14 (4)
Zaprinast, 0.1 mM	3.72 ± 0.23* (8)	3.27 ± 0.20 (4)	1.29 ± 0.16** (8)	1.19 ± 0.07 (4)
+ 50 mM K^+		3.59 ± 0.21* (4)		2.12 ± 0.44* (4)
ODQ, 0.01 mM	3.74 ± 0.27* (8)	3.36 ± 0.21 (4)	2.18 ± 0.14 (7)	2.22 ± 0.07** (4)
+ 50 mM K^+		3.35 ± 0.14* (4)		3.58 ± 0.29 (4)
PMA, 0.00001 mM	3.84 ± 0.12** (8)	3.17 ± 0.20 (4)	1.36 ± 0.08** (6)	1.48 ± 0.18 (4)
+ 50 mM K^+		3.77 ± 0.25 (4)		3.20 ± 0.04 (4)
Chelerythrine, 0.001 mM	4.97 ± 0.47 (6)	3.13 ± 0.19 (4)	1.68 ± 0.19 (6)	1.30 ± 0.11 (4)
+ 50 mM K^+		5.42 ± 0.45 (4)		3.59 ± 0.30 (4)
H-7, 0.1 mM	5.18 ± 0.42 (7)	3.27 ± 0.18 (4)	1.29 ± 0.10** (7)	0.92 ± 0.15 (4)
+ 50 mM K^+		5.16 ± 0.45 (4)		2.41 ± 0.19** (4)

The drugs were added at the beginning of superfusion and 50 mM K^+ at 30 min. The results show the efflux rate constants ± SEM ($\times 10^{-3}$ min^{-1}) for the time intervals of 20–30 min (k_1) and 32–40 min (k_2) with the number of independent experiments in parenthesis. Significance of differences from the corresponding controls: $^*p < 0.05$, $^{**}p < 0.01$

IBMX, 3-isobutyl-1-methylxanthine; *ODQ*, 1H-(1,2,4)oxadiazolo(4,3-a)guinoxalin-1-one; *IBMX*, 3-isobutyl-1-methylxanthine; *PMA*, 4β-phorbol 12-myristate 13-acetate; *H-7*, 1-(5-isoquinolinesulphonyl)-2-methylpiperazine

generation of high-energy phosphate reserves is then not efficient enough to maintain cellular ionic gradients and other metabolic processes. In ischemia neurons are suddenly depolarized. This event is accompanied by an increase in the extracellular K^+ concentration, a decrease in extracellular Na^+ levels (Somjen et al., 1990) and a massive release of glutamate. Simultaneously, an extracellular accumulation of taurine has been demonstrated in different brain regions (Hagberg et al., 1985; Globus et al., 1988; Shimada et al., 1993; Saransaari and Oja, 1997a, 2000a). The release of GABA is also enhanced under cell-damaging conditions, including ischemia, in other brain areas (Globus et al., 1988; Shimada et al., 1993; Saransaari and Oja, 1997b, 1998b) and the brain stem (Saransaari and Oja, 2005). These releases might be important protective mechanisms against the excessive release of excitatory amino acids in the brain stem, counteracting harmful effects that lead to neuronal death.

We have extensively studied the properties of ischemia-induced release of taurine in the mature and immature mouse hippocampus (Saransaari and Oja, 1997a, 1998a, 1999, 2000a, b, 2002a, b). As in normoxia (Saransaari and Oja, 2006) these multiple mechanisms are apparently also involved in the ischemia-induced taurine release in the brain stem. The marked increase in release under cell-damaging conditions could be due to several mechanisms, including Ca^{2+} -dependent exocytosis, Ca^{2+}-independent release via reversal carrier-mediated uptake, indiscriminate opening of ion channels and unspecific leakage through damaged plasma membranes. Depolarization by K^+ stimulation was now able to further potentiate the ischemic taurine release at both ages. The absence and reduction of K^+ stimulation in the absence of Ca^{2+} in the adult and developing brain stem, respectively, indicates that at least a part of the release is clearly Ca^{2+}-dependent, as in the mouse hippocampus (Saransaari and Oja, 1998a, 1999). In normoxia the evoked release has

also been partially Ca^{2+}-dependent (Saransaari and Oja, 1998a, 1999, 2006). Even though Ca^{2+}-dependent processes may thus be involved, the release could also result from excitotoxicity-induced cellular swelling under cell-damaging conditions. Both potassium ion depolarization (Oja and Saransaari, 1992) and exposure to glutamate receptor agonists (Saransaari and Oja, 1991) have been shown to induce swelling-associated release of taurine in brain slices. The Ca^{2+}-dependent exocytosis of synaptic vesicles may thus play a minor role in the ischemia-evoked release in the brain stem.

A regulatory volume decrease has also been held to contribute to glutamate and aspartate release during ischemia (Pasantes-Morales, 1996). In this process swollen cells attempt to regain their normal volume by releasing osmolytes, including taurine. The swelling-induced increase in taurine release has been demonstrated to be a diffusional process without any involvement plasma membrane carriers (Sánchez-Olea et al., 1993). The chloride channel antagonists DIDS and SITS now reduced the ischemia-induced taurine release, indicating that this release may occur through anion channels, similarly to the volume-sensitive taurine release in astrocytes and neurons (Schousboe et al., 1991) and ischemic taurine release in the hippocampus (Saransaari and Oja, 1998a, 1999).

The ischemia-induced taurine release in the brain stem was stimulated by taurine itself and also by the structural analogue GABA, suggesting that both homo- and hetero-exchange are operating in this situation. Furthermore, the release in developing mice was increased by Na^+ deficiency, as in other brain regions (see Saransaari and Oja, 1992). Na^+-free medium is known to diminish the K^+ content of the slices (Korpi and Oja, 1983), due to inhibition of Na^+, K^+-ATPase. This bespeaks the involvement of Na^+-dependent taurine transporters operating outwards. Indeed, brain tissue possesses a saturable Na^+-requiring transport system for taurine at neuronal and glial cell membranes, comprising both high- and low-affinity components (Oja and Kontro, 1984; Holopainen et al., 1987). The cloned taurine transporter has been localized in the brain stem in the mouse (Liu et al., 1992) and could exhibit this kind of behavior. Furthermore, taurine is able to interact with GABA transporters (GAT, Palacín et al., 1998; Sivakami et al., 1992). GAT 1, 2 and 3 are all expressed in the brain stem on neuronal and glial membranes (López-Corcuera et al., 1992; Palacín et al., 1998). The taurine transporter also exhibits profound Cl^--dependence (Smith et al., 1992). When the Na^+-gradient is dissipated, the preferred direction of transport changes from inward (uptake) to outward (release). The involvement of transporters in the release in the immature mice was further confirmed by the effects of taurine and GABA. Under Na^+-free conditions the stimulation by these amino acids was not discernible, the carriers not being functional without Na^+. The results clearly demonstrate that Na^+-dependent transporters mediate a considerable part of the Ca^{2+}-independent taurine release in ischemia. In keeping with this, taurine transporters in the mouse cerebral cortex are found to be still operative in ischemia, though non-saturable diffusion is also concomitantly greatly increased (Saransaari and Oja, 1996).

Activation of presynaptic protein kinase C (PKC) has enhanced the release of neurotransmitters from nerve terminals (Barrie et al., 1991; Eboli et al., 1993) and protein kinases/phosphatases may also play a role in cell volume regulation (Grinstein et al., 1992). PKC has enhanced taurine release in mouse hippocampal slices in normal conditions (Saransaari and Oja, 2002a) and in the rat cortex in vivo during hyposmotic stress (Estevez et al., 1999), whereas the taurine transporter has been shown to be regulated by activation of both protein kinases A and C in *Xenopus* oocytes (Loo et al., 1996). The PKC activator PMA or inhibitor chelerythrine have been ineffective on taurine release in mouse brain stem (Saransaari and Oja, 2006) and in glial cells from the rat supraopticus nuclei (Deleuze et al., 2000). However, PKC has been thought to play a role in the ischemia-induced amino acid release, taurine release being attenuated by the protein kinase inhibitor H-7 in the striatum of hypertensive rats (Nakane et al., 1998). Also the reduction of ischemic taurine release by PMA at both ages in the brain stem indicates the involvement of PKC. On the other hand, in the hippocampus the ischemic taurine release was not dependent on protein kinase C, since both H-7 and tamoxifen had no effects on the release (Saransaari and Oja, 2002a). Tyrosine kinases seem to have only a small effect on release in the brain stem, since the inhibitor genistein reduced the stimulated efflux only in adult mice. However, the involvement of tyrosine kinases in taurine release induced by the hypo-osmotic stress has been thought to obtain in various cell types, including brain cells, on the basis of the effects of tyrosine kinase and phosphatase blockers (Deleuze et al., 2000; Morales-Mulia et al., 2001).

The rise in cyclic guanosine monophosphate (cGMP) levels evokes taurine release in the hippocampus under both normal and ischemic conditions (Saransaari and Oja, 2002a). In the brain stem, ODQ, an inhibitor of soluble guanylyl cyclase, as well as all phosphodiesterase inhibitors (IBMX, RO 20-1724, and zaprinast) generally now

reduced the ischemic taurine release, suggesting that increased cGMP levels inhibit taurine release. On the other hand, cyclic adenosine monophosphate (cAMP) may not be involved because alloxan, an inhibitor of cyclic adenylyl cyclase, had no effect. This is at variance with the results obtained under normal conditions, where cAMP has been able to modify taurine release in the brain stem (Saransaari and Oja, 2006).

Phospholipase-induced plasma membrane disruption has been suggested to be involved in the ischemia-evoked release of neurotransmitter amino acids in the cerebral cortex (O'Regan et al., 1995; Phillis and O'Regan, 2003). Topical application of phospholipase A_2 has elicited a profound increase in the cortical superfusate levels of taurine (Phillis et al., 1997). Volume-activated taurine release in hippocampal slices (Franco et al., 2001) and in the rat brain in vivo (Estevez et al., 1999) has been insensitive to phospholipase inhibitors. On the other hand, under normal conditions in the developing and adult mouse hippocampus (Saransaari and Oja, 1998a, 1999) the phopholipase A_2 inhibitor quinacrine has potentiated taurine release, indicating that other mechanisms than membrane disruption participate. However, both under normal (Saransaari and Oja, 2006) and ischemic conditions (present study) taurine release in the brain stem is markedly inhibited the phospholipase inhibitor by guinacrine, indicating the participation of phospholipases on taurine release in this brain area.

In conclusion, the release of taurine from the brain stem from 3-month-old and 7-day-old mice was markedly enhanced under cell-damaging conditions, including hypoxia, hypoglycemia, ischemia and metabolic inhibition (DNP). The ischemia-induced taurine release consisted of both Ca^{2+}-dependent and Ca^{2+}-independent components. Moreover, the release was mediated by Na^+-, Cl^--dependent transporters operating outwards and partly by chloride ion channels. PKC seems to be involved in ischemic taurine release in the brain stem and the release is also modulated by the cGMP second messenger systems and phospholipases at both ages. Similarly to the hippocampus, the release of taurine induced by cell-damaging conditions may provide an important protective mechanism against excitotoxicity and neural cell damage in the brain stem.

Acknowledgements

The skilful technical assistance of Mrs. Irma Rantamaa and Mrs. Oili Pääkkönen and the financial support of the competitive research funding of the Pirkanmaa Hospital District are gratefully acknowledged.

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