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ORIGINAL ARTICLE

Modulation of taurine release in ischemia by glutamate receptors in mouse brain stem slices

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Abstract Glutamate is the main excitatory transmitter in the brain stem, regulating many vital sensory and visceral processes. Taurine is inhibitory and functions as a neuromodulator and regulator of cell volumes in the brain, being especially important in the developing brain. Taurine release is markedly enhanced under ischemic conditions in many brain areas, providing protection against excitotoxicity. The involvement of glutamate receptors in the release of preloaded [^{3}H]taurine was now characterized under ischemic conditions in slices prepared from the mouse brain stem from developing (7-day-old) and young adult (3-month-old) mice. The ionotropic glutamate receptor agonists *N*-methyl-D-aspartate, kainate, and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate had no effect on ischemic taurine release in the immature brain stem, whereas in adults the release was enhanced in a receptor-mediated manner. The metabotropic receptor agonists of group I, (1±)-1-aminocyclopentane-*trans*-1,3-dicarboxylate and (*S*)-3,5-dihydroxyphenylglycine, potentiated both basal and K^+-stimulated release in both age groups. The group III agonist L(+)-2-amino-4-phosphonobutyrate also enhanced the release. In both cases the effects were receptor-mediated, being reduced by the respective antagonists. The results show that activation of glutamate receptors in the ischemic brain stem generally enhances the release of taurine. This is beneficial to neurons in ischemia, offering protection against excitotoxicity and preventing neuronal damage.

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Abbreviations

t-ACPD	(1±)-1-aminocyclopentane-*trans*-1,3-dicarboxylate
2*R*,4*R*-APDC	(2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate
AIDA	(*RS*)-1-aminoindan-1,5-dicarboxylate
AMPA	2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CPPG	(*RS*)-2-cyclopropyl-4-phosphonophenylglycine
DHPG	(*S*)-3,5-dihydroxyphenylglycine
EGLU	2*S*-2-ethylglutamate
Hepes	*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid
L-AP4	L(+)-2-amino-4-phosphonobutyrate
L-SOP	*O*-phospho-L-serine
mGluR	Metabotropic glutamate receptor
MK-801	Dizocilpine [(5*S*, 10*R*)-(+)-methyl-10,11-dihydro-5*H*-dibenzo(a,d)cyclohepten-5,10-amine, MK-801]
NBQX	2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-[f]quinoxaline-7-sulfonamide
NMDA	*N*-methyl-D-aspartate
QA	Quisqualate

Introduction

The brain stem is the most important region in the regulation of many sensory and visceral processes, e.g., the location of the cardiovascular and respiratory centers.

Glutamate has been implicated as the pivotal excitatory transmitter in the central nervous system, including the brain stem, both ionotropic and metabotropic glutamate receptors being involved in a diverse array of functions (Pierrefiche et al. 1994). The three types of ionotropic receptors are located in brain stem nuclei (Liu and Wong-Riley 2004), which also contain three groups of metabotropic glutamate receptors (mGluRs) (Azcue et al. 1997; de Novellis et al. 2003).

The inhibitory amino acid taurine has been thought to function as a regulator of neuronal activity, inducing hyperpolarization and inhibiting firing of central neurons (Oja and Kontro 1983a; Saransaari and Oja 1992). It is also involved in osmoregulation and cell volume adjustments in the central nervous system (Oja and Saransaari 1996). Furthermore, taurine has a special role in immature brain tissue (Oja and Kontro 1983a; Kontro and Oja 1987; Sturman 1993), being apparently essential for the development and survival of neural cells (Sturman 1993). Taurine protects neural cells from excitotoxicity induced by excitatory amino acids in different brain areas (Trenkner 1990; Tang et al. 1996), prevents harmful metabolic responses evoked by ischemia (Schurr et al. 1987), and alleviates symptoms in epilepsy (Oja and Kontro 1983b). Moreover, taurine has recently been shown to prevent ischemia-induced apoptosis (Takatani et al. 2004; Taranukhin et al. 2008). The anti-apoptotic function has been proposed to be due to inhibition of glutamate-induced membrane depolarization (Leon et al. 2008).

The levels of taurine in the brain stem are not so high as in higher brain regions, but, for example, taurine abounds in the lateral geniculate nucleus, inferior colliculus, and auditory brain stem (see Oja and Kontro 1983a), as well as in the periaqueductal grey, medullary nucleus raphe magnus and spinal trigeminal nucleus (Renno et al. 2008). Taurine has been suggested to be involved in the ventrolateral medulla in modulation of cardiovascular control (Kubo et al. 1993; Wang et al. 2005) and in the nucleus of the solitary tract in neurogenic control of arterial blood pressure (Meeley et al. 1989). Taurine is released from the locus coeruleus by various stimuli (Singewald and Philippu 1998), and there is also evidence that the released taurine therein is involved in conditioned fear (Kaehler et al. 2000). We have recently systematically characterized the properties of taurine release in brain stem slices from both adult and developing mice (Saransaari and Oja 2006, 2007, 2008). The release, consisting of both Ca^{2+}-dependent and -independent components and mediated to a part by Na^{+} and Cl^{-}-dependent transporters, was markedly enhanced under ischemic conditions, as in higher brain areas (Saransaari and Oja 1992, 1997a, 1998a, b, 1999a; Phillis and O'Regan 2003). It thus seems to provide protection against excitotoxicity (Saransaari and Oja 2000a). Now we characterized under ischemic conditions the involvement of glutamate receptors in the release of preloaded [^{3}H]taurine in slices prepared from the mouse brain stem from developing (7-day-old) and young adult (3-month-old) mice, using a superfusion system.

Materials and methods

Materials

Young adult (3-month-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. (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-APDC), (*RS*)-1-aminoindan-1,5-dicarboxylate (AIDA), 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (*RS*)-2-cyclopropyl-4-phosphonophenylglycine (CPPG), (*S*)-3,5-dihydroxyphenylglycine (DHPG), 2*S*-2-ethylglutamate (EGLU), L(+)-2-amino-4-phosphonobutyrate (L-AP4), *O*-phospho-L-serine (L-SOP), dizocilpine [(5*S*, 10*R*)-(+)-methyl-10,11-dihydro-5*H*-dibenzo(a,d)cyclohepten-5,10-amine, MK-801], 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-[f]quinoxaline-7-sulfonamide (NBQX), *N*-methyl-D-aspartate (NMDA), quisqualate (QA), and (1±)-1-amino-cyclopentane-*trans*-1,3-dicarboxylate (t-ACPD) were from Tocris Bioscience (Bristol, UK). Other reagents and drugs were from common commercial sources.

Release experiments

Superficial slices 0.4 mm thick and 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 standard medium (unless otherwise specified) at a rate of 0.25 ml/min for 50 min in a system in which freely floating shaken slices were kept under a continuous flow of oxygen 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 superfusion the slices were weighed, homogenized in ice-cold 5% (w/v) trichloracetic acid solution, centrifuged, and the clear supernatants used for scintillation counting. The effluent samples were subjected to the same analyses.

Superfusion conditions

Ischemic conditions were induced by modified experimental conditions: the glucose-free medium was bubbled with N_2 gas (Taylor et al. 1995) for 30 min before the experiments and then during the whole superfusion.

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, initial release phase) and 32–40 min (k_2, later release phase) were computed as negative slopes for the regression lines of the logarithm of radioactivity remaining in the slices versus superfusion time. In many experiments, the medium was replaced with another (K^+ 50 mM, antagonists) at 30 min and rate constants were then calculated for the time interval of 32–40 min.

Statistical analyses

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

Results

Adult 3-month-old mice

The release rate of [^{3}H]taurine induced by ischemia in 3-month-old mice was initially high, then decreasing rather steeply, as shown by the efflux rate constants k_1 and k_2 (Table 1). K^+ stimulation (50 mM) did not significantly enhance the release (Table 1). When added to superfusion medium at 30 min, glutamate (0.1 mM) had no significant effect on basal taurine release, while QA, kainate, and AMPA enhanced it (Fig. 1). The K^+-stimulated release was not affected (data not shown). The effects of AMPA and kainate were reduced, but not abolished, by the antagonists NBQX (0.1 mM) and CNQX (0.1 mM), respectively (Fig. 1). QA, NMDA, kainate, and AMPA when added at the beginning of superfusions potentiated the basal release in ischemia (k_2) (Table 1). The effect of kainate was abolished by CNQX ($P < 0.01$) and that of AMPA by NBQX ($P < 0.05$). NMDA was no longer stimulatory in the presence of the antagonist dizocilpine (MK-801). AMPA stimulated and NBQX reduced ($P < 0.05$) the initial superfusion rate (k_1) (Table 1). The K^+-stimulated release was enhanced by glutamate and AMPA, the AMPA effect being unaffected by NBQX (Table 1). MK-801, CNQX, and NBQX alone had no effect on the releases (data not shown).

The mGluR receptor agonists were in all cases added at the beginning of superfusions. In adult mice, the group I agonist t-ACPD had no effect on the release, whereas

Table 1 Effects of the agonists and antagonists of ionotropic glutamate receptors on taurine release from brain stem slices from 3-month-old mice in ischemia

Effector (0.1 mM)	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM		
	k_1	k_2	k_2 (50 mM K^+)
Basal (control)	4.29 ± 0.24 (37)	3.16 ± 0.23 (10)	3.71 ± 0.20 (4)
Glutamate	4.98 ± 0.36 (7)	3.96 ± 0.12 (4)	5.88 ± 0.45** (4)
QA	4.25 ± 0.28 (4)	4.13 ± 0.41* (4)	4.23 ± 0.11 (4)
NMDA	5.06 ± 0.30 (15)	4.59 ± 0.45** (4)	4.35 ± 0.46 (4)
+MK-801		3.38 ± 0.33 (4)	–
Kainate	4.52 ± 0.19 (8)	4.36 ± 0.11** (4)	4.32 ± 0.14 (4)
+CNQX		2.84 ± 0.14[b] (4)	–
AMPA	5.76 ± 0.23* (5)	4.67 ± 0.30** (4)	4.78 ± 0.21* (4)
+NBQX	4.85 ± 0.23[a] (4)	3.62 ± 0.23[a] (4)	5.28 ± 0.25** (4)

The slices were preloaded for 30 min with 10 μM [^{3}H]taurine in Krebs-Ringer-Hepes-glucose medium, pH 7.4, and then superfused for 50 min. The ionotropic glutamate receptor agonists and the antagonist NBQX were present from the beginning of superfusions, while the antagonists MK-801 and CNQX were added at 30 min. The results are efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM. Number of independent experiments is provided in parentheses

Significance of differences from the control: * $P < 0.05$, ** $P < 0.01$. Significance of differences between an agonist and the corresponding antagonist: [a]$P < 0.05$, [b]$P < 0.01$

NMDA, *N*-methyl-D-aspartate; *MK-801*, dizocilpine (5*S*, 10*R*)-(+)-methyl-10,11-dihydro-5*H*-dibenzo(a,d)cyclohepten-5,10-amine; *NBQX*, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; *CNQX*, 6-cyano-7-nitroquinoxaline-2,3-dione; *QA*, quisqualate; *AMPA*, 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate

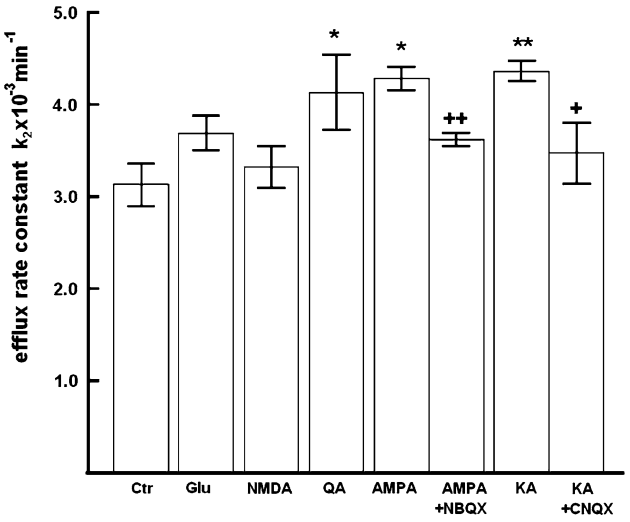

Fig. 1 Release of [^{3}H]taurine from brain stem slices from 3-month-old mice under ischemic conditions in the presence of different ionotropic glutamate receptor agonists and antagonists (all 0.1 mM). The results show the efflux rate constants (±SEM) k_2 (32–40 min), mean values of 4–8 independent experiments. Significance of differences from the control: *$P < 0.01$ and significance of differences between an agonist and the antagonist: +$P < 0.05$, ++$P < 0.01$. *NMDA* *N*-methyl-D-aspartate, *QA* quisqualate, *AMPA* 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate, *NBQX* 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-[f]quinoxaline-7-sulfonamide, *KA* kainate, *CNQX* 6-cyano-7-nitroquinoxaline-2,3-dione

the other group I agonist DHPG (0.1 mM) enhanced the later phase of basal release (k_2) and the K^+-stimulated release (Table 2). This latter enhanced release was reduced by the antagonist AIDA (0.1 mM) (Table 2). The group II agonist 2*R*,4*R*-APDC (0.1 mM) had no effect. The basal release (k_2) was not affected by the group III agonist L-SOP (0.1 mM), while the agonist L-AP4 (0.1 mM, group III) enhanced it significantly; this effect being abolished by the antagonist CPPG (0.1 mM). Both group III agonists likewise potentiated the K^+-evoked release, but these effects were not significantly altered by CPPG (Table 2). AIDA, EGLU, and CPPG alone had no effects (data not shown).

Developing 7-day-old mice

The release of taurine in ischemia in 7-day-old mice was fairly steady during the whole superfusion period (Fig. 2; Table 3). It was increased about twofold by K^+ stimulation (Table 3). When added to superfusion medium at 30 min, glutamate (0.1 mM) had no significant effect on basal taurine release (data not shown). Also, QA, the ionotropic glutamate receptor agonists kainate, NMDA, and AMPA (all 0.1 mM) did not affect the basal or K^+-stimulated release (data not shown). When the substances were added to superfusion medium at the beginning of efflux experiments, glutamate, NMDA, kainate, and AMPA had no effects (data not shown), but QA markedly increased ($P < 0.05$) the initial release phase (k_1) to $2.40 \pm 0.20 \times 10^{-3}$ min^{-1} (mean ± SEM, $n = 7$) when compared to the control phase $1.58 \pm 0.13 \times 10^{-3}$ min^{-1} ($n = 55$).

Table 2 Effects of agonists and antagonists of metabotropic glutamate receptors on taurine release from brain stem slices from 3-month-old mice in ischemia

Effector (0.1 mM)	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM		
	k_1	k_2	k_2 (50 mM K^+)
Basal (control)	4.29 ± 0.24 (37)	3.16 ± 0.23 (10)	3.71 ± 0.20 (4)
t-ACPD	5.37 ± 0.33 (7)	3.75 ± 0.31 (4)	4.56 ± 0.33 (8)
+AIDA		2.70 ± 0.19 (4)	4.20 ± 0.15 (4)
DHPG	5.14 ± 0.15 (14)	4.30 ± 0.47* (4)	5.18 ± 0.32* (7)
+AIDA		3.60 ± 0.34 (8)	4.19 ± 0.28[a] (7)
2*R*,4*R*-APDC	5.12 ± 0.31 (12)	3.52 ± 0.29 (4)	4.81 ± 0.26 (7)
+EGLU		3.78 ± 0.16 (7)	4.44 ± 0.36 (8)
L-AP4	4.81 ± 0.22 (16)	4.02 ± 0.14* (4)	5.00 ± 0.37* (4)
+CPPG		2.75 ± 0.04[b] (4)	4.35 ± 0.18 (4)
L-SOP	4.48 ± 0.26 (21)	3.05 ± 0.39 (4)	5.04 ± 0.12** (4)
+CPPG		3.34 ± 0.19 (4)	4.52 ± 0.26 (8)

The slices were preloaded for 30 min with 10 μM [^{3}H]taurine in Krebs-Ringer-Hepes-glucose medium, pH 7.4, and then superfused for 50 min. The metabotropic glutamate receptor agonists were present from the beginning of superfusions, while the antagonists were added at 30 min. The results are efflux rate constants ($\times 10^{-3}$ min^{-1}) ±SEM. Number of independent experiments is given in parentheses

Significance of differences from the control: * $P < 0.05$, ** $P < 0.01$. Significance of differences between an agonist and the corresponding antagonist: [a]$P < 0.05$, [b]$P < 0.01$

t-ACPD, (1±)-1-aminocyclopentane-*trans*-1,3-dicarboxylate; *DHPG*, (*S*)-3,5-dihydroxyphenylglycine; *AIDA*, (*RS*)-1-aminoindan-1,5-dicarboxylate; *2R,4R-APDC* (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate; *EGLU*, (2*S*)-2-cyclopropyl-4-phosphonophenylglycine; *CPPG*, (*RS*)-2-cyclopropyl-4-phosphonophenylglycine; *L-AP4*, L(+)-2-amino-4-phosphonobutyrate; *L-SOP*, *O*-phospho-L-serine

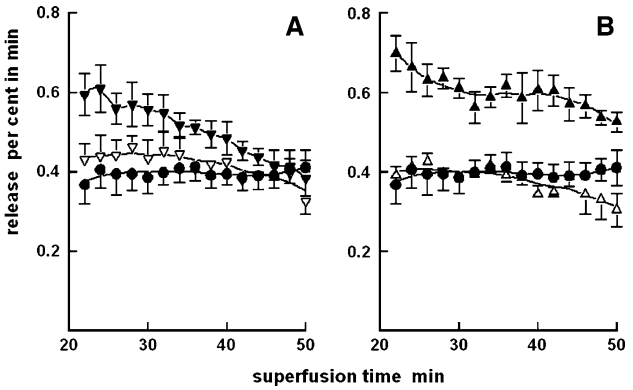

Fig. 2 Release of [^{3}H]taurine from brain stem slices from 7-day-old mice in ischemia **a** control (*filled circle*), in the presence of 0.1 mM t-ACPD (*filled triangle*) and 0.1 mM t-ACPD with 0.1 mM AIDA (*open triangle*), **b** control (*filled circle*), in the presence of 0.1 mM L-AP4 (*filled triangle*) and 0.1 mM L-AP4 with 0.1 mM CPPG (*open triangle*). *t-ACPD* (1±)-1-aminocyclopentane-*trans*-1,3-dicarboxylate, *AIDA* (*RS*)-1-aminoindan-1,5-dicarboxylate, *L-AP4* L(+)-2-amino-4-phosphonobutyrate, *CPPG* (*RS*)-2-cyclopropyl-4-phosphonophenylglycine

In developing mice, t-ACPD enhanced both the initial (k_1) (Fig. 2a) and K^+-stimulated (Table 3) release. Both these effects were reduced by AIDA when it was already added at the beginning of superfusion. The other group I agonist tested DHPG had no effect. The agonist 2*R*,4*R*-APDC potentiated the initial release; this effect was not abolished by the antagonist EGLU (0.1 mM). The release was not markedly affected by L-SOP but enhanced (k_1) by L-AP4 (Fig. 2b). The effect of L-AP4 was abolished by CPPG added at the onset (Fig. 2b). CPPG likewise reduced the release in the presence of L-SOP (Table 3). AIDA, EGLU, and CPPG alone had no effect on the releases (data not shown).

Discussion

Basal and K^+-stimulated taurine releases are markedly enhanced under ischemic conditions in the brain stem in both adult and developing mice, the effects being greater in the latter. These releases seem to be regulated by both ionotropic and metabotropic glutamate receptors, as also in higher brain areas (Saransaari and Oja 1997b, c, 1999b, 2000b). However, this modulation is somewhat different when compared with that under normal conditions, and marked differences were also obtained between the mature and immature brain stem.

Ionotropic glutamate receptor agonists failed now to have any effects on taurine release in ischemia in the developing brain stem, whereas under normal conditions NMDA and AMPA receptors are involved in the release (Saransaari and Oja 2006). In general, the ability of the ionotropic glutamate receptor agonists to evoke taurine release under cell-damaging conditions has been stronger in the developing than in the adult brain (Saransaari and

Table 3 Effects of agonists and antagonists of metabotropic glutamate receptors on taurine release from brain stem slices from 7-day-old mice in ischemia

Effector (0.1 mM)	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM		
	k_1	k_2	k_2 (50 mM K^+)
Basal (control)	1.58 ± 0.13 (55)	1.74 ± 0.27 (11)	3.30 ± 0.27 (4)
t-ACPD	2.72 ± 0.22** (6)	1.96 ± 0.18 (4)	5.28 ± 0.44** (4)
+AIDA	1.80 ± 0.09[b] (15)	1.75 ± 0.10 (8)	3.20 ± 0.10[b] (8)
DHPG	2.07 ± 0.23 (8)	1.99 ± 0.17 (4)	3.64 ± 0.22 (4)
+AIDA	2.35 ± 0.20 (4)	2.16 ± 0.18 (4)	–
2*R*,4*R*-APDC	2.53 ± 0.15* (5)	2.29 ± 0.19 (4)	3.46 ± 0.31 (4)
+EGLU	2.97 ± 0.15** (4)	2.84 ± 0.11 (4)	–
L-AP4	2.95 ± 0.34** (6)	2.60 ± 0.21 (4)	3.05 ± 0.19 (4)
+CPPG	1.67 ± 0.22[a] (4)	1.39 ± 0.19[b] (4)	–
L-SOP	2.11 ± 0.13 (5)	2.33 ± 0.18 (4)	3.58 ± 0.19 (4)
+CPPG	1.44 ± 0.14[a] (4)	1.54 ± 0.17[a] (4)	–

The slices were preloaded for 30 min with 10 μM [^{3}H]taurine in Krebs-Ringer-Hepes-glucose medium, pH 7.4, and then superfused for 50 min. The metabotropic glutamate receptor agonists and the antagonists were present from the beginning of superfusions. The results are efflux rate constants ($\times 10^{-3}$ min^{-1}) ±SEM. Number of independent experiments is provided in parentheses

Significance of differences from the control * $P < 0.05$, ** $P < 0.01$. Significance of differences between an agonist and the corresponding antagonist: [a]$P < 0.05$, [b]$P < 0.01$

t-ACPD, (1 ±)-1-aminocyclopentane-*trans*-1,3-dicarboxylate; *DHPG*, (*S*)-3,5-dihydroxyphenylglycine; *AIDA*, (*RS*)-1-aminoindan-1,5-dicarboxylate; *2R,4R-APDC*, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate; *EGLU*, (2*S*)-2-cyclopropyl-4-phosphonophenylglycine; *CPPG*, (*RS*)-2-cyclopropyl-4-phosphonophenylglycine; *L-AP4*, L(+)-2-amino-4-phosphonobutyrate, and *L-SOP*, *O*-phospho-L-serine

Oja 1997b, c, 2003), providing a means to protect neural cells against excitotoxicity. This mechanism appears to be absent in the developing brain stem, as NMDA, AMPA, and kainate were only effective in adults. The actions of AMPA and kainate were reduced by their respective antagonists, suggesting that their receptors are involved. This mechanism may be important in the adult brain stem, as the ionotropic glutamate receptor agonists have failed to have any effects on the release of the other inhibitory amino acid GABA (Saransaari and Oja 2005). High concentrations of excitatory amino acids are neurotoxic, and overstimulation of the ionotropic glutamate receptors contributes to neuronal death during cerebral ischemia (Szatkowski and Attwell 1994; Ozawa et al. 1998). These amino acids are massively released from neural structures in hypoxia and ischemia, as has been demonstrated both in vitro (Collard and Menon-Johansson 1993; Saransaari and Oja 1998b, Oja and Saransaari 2009) and in vivo (O'Regan et al. 1989). The activation of NMDA receptors in particular triggers a cascade of cellular events, increasing the intracellular concentration of Ca^{2+} and potentiating NMDA-gated currents, which culminates in neuronal cell death (Szatkowski and Attwell 1994; Ozawa et al. 1998).

Metabotropic glutamate receptors have also been held to be involved in both the potentiation and the prevention of excitotoxic and ischemic neuronal damage (Conn and Pin 1997; Ozawa et al. 1998). Drugs acting on these receptors have generally reduced taurine release in the brain stem in normoxia (Saransaari and Oja 2006), but now tended to enhance the release in ischemia. This is in harmony with the fact that the activation of mGluRs can have both excitatory and inhibitory effects on neuronal activity, depending on circumstances (Ozawa et al. 1998). The observed variability in the responses of taurine release to metabotropic glutamatergic agents stems from the receptor heterogeneity, from the considerable overlap in their selectivity for the drugs used (Conn and Pin 1997), and from the possible cross-talk between the different receptor subtypes (Schaffhauser et al. 1997). An agonist can either stimulate two groups of receptors or act as an agonist for one group and as an antagonist for the other. The mGluR agonists have not been seen to affect basal and K^+-stimulated taurine release in normoxia in the adult brain stem (Saransaari and Oja 2006). Now in ischemia, the agonists enhanced both types of release. The present potentiating influence of t-ACPD (in developing mice) and DHPG (in adults) is apparently mediated through group I mGluRs, as both effects were reduced by the antagonist AIDA. The level of $mGluR_1$ expression is fairly high in brain stem nuclei and increases gradually during early postnatal development concomitant with the maturation of neuronal elements (Shigemoto et al. 1992). The expression of $mGlu_{5a}$-receptor mRNA is higher during early postnatal life than in adults, where the $mGlu_{5b}$-receptor mRNA is predominant (Minakami et al. 1995). The mGluRs of group I have been shown to synergize with NMDA receptors in inducing neuronal damage (McDonald and Schoepp 1992; Strasser et al. 1998). QA and DHPG also enhance NMDA toxicity in cultured neurons (Buisson and Choi 1995), which would suggest that activation of group I mGluRs contributes to ischemic brain damage. Furthermore, excitotoxic damage in a hippocampal model of ischemia has been thought to be caused by the release of endogenous glutamate due to activation of group I mGluRs by DHPG and *trans*-ACPD (Strasser et al. 1998). Thus, taurine release enhanced by mGluR I activation by DHPG and t-ACPD in both adult and developing brain stem may reduce hyperexcitation and strengthen inhibitory effects, being thus neuroprotective.

The agonist of group II mGluR 2*R*,4*R*-APDC stimulated the ischemia-induced taurine release in developing mice, but the effect was not affected by the antagonist EGLU. The expressions of mRNA for mGluR2 and mGluR3 have generally been moderate or low in the brain stem (Ohishi et al. 1993a, b). On the other hand, the potentiations by L-AP4, a group III agonist, at both ages were reduced by the antagonist CPPG, indicating the involvement of group III mGluRs. The expression of L-AP4 sensitive mGluR has been localized by in situ hydridization in different brain areas, including brain stem nuclei (Ohishi et al. 1995). It has been suggested that group II and III mGluRs function as inhibitory autoreceptors (Sánchez-Prieto et al. 1996). The agonists of both group II and group III receptors are thus neuroprotective. The agonists of group III mGluRs L-AP4 and L-SOP produce neuroprotective effects in cultured neurons and brain slices (Bruno et al. 1995), noncompetitively inhibit NMDA-induced toxicity in cerebellar granule neurons (Lafon-Cazal et al. 1999), and the Ca^{2+}-dependent release of glutamate is reduced by L-AP4 in a concentration-dependent manner (Herrero et al. 1996). It is thus apparent that the enhanced taurine release induced by group II and III mGluRs is beneficial to neurons in ischemia, corroborating the assumption of neuroprotective effects of these receptors in the brain stem. Moreover, it is of note that the ischemia-induced K^+-stimulated release was also potentiated by these receptor agonists. The situation seems to be different in higher brain areas, for example, in the hippocampus, where the release in ischemia has been suppressed by mGluR agonists in adults, while the group I agonists DHPG and trans-ACPD have enhanced the release in developing mice (Saransaari and Oja 2000b).

In conclusion, the release of taurine induced by ischemic conditions is modulated by both ionotropic and metabotropic glutamate receptor agonists in the adult and

developing mouse brain stem. The release is enhanced by ionotropic AMPA and kainate agonists in a receptor-mediated manner in adults. On the other hand, the basal and K^+-stimulated releases are potentiated by the mGluR agonists t-ACPD, DHPG (group I), and L-AP4 (group II) in both the mature and the immature brain stem. It thus appears that activation of glutamate receptors in the ischemic brain stem generally enhances the release of inhibitory taurine. This is beneficial to neurons in ischemia, exerting neuroprotective effects and preventing excitotoxic neuronal damage.

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