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ORIGINAL ARTICLE

Modulation of taurine release in glucose-free media by glutamate receptors in hippocampal slices from developing and adult mice

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Abstract Taurine has been thought to protect neural cells against cell-damaging conditions to which the hippocampus is particularly vulnerable. We studied now how the release of preloaded [^{3}H]taurine is regulated by glutamate receptors in glucose-free media in slices prepared from the mouse hippocampus from developing (7 days old) and young adult (3 months old) mice, using a superfusion system. The lack of glucose enhanced taurine release more from slices from developing mice than from slices from adults. At both ages ionotropic glutamate agonists significantly increased the release in a receptor-mediated manner. Of the metabotropic glutamate receptors those belonging to the group III were effective. The release was enhanced in adult mice but attenuated in developing mice. Both effects were blocked by the receptor antagonists. The results show that glutamate receptors affect taurine release in the absence of glucose in which condition taurine should be neuroprotective.

Keywords Taurine release · Glutamate receptors · Hippocampus · Tissue slices · Adults · Developing mice

Abbreviations

2R,4R-APDC	(2R,4R)-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	2S-2-Ethylglutamate
Hepes	*N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid
L-AP4	L(+)-2-Amino-4-phosphonobutyrate
L-SOP	*O*-Phospho-L-serine
MK-801	Dizocilpine [(5S, 10R)-(+)-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
t-ACPD	(1±)-1-Aminocyclopentane-*trans*-1,3-dicarboxylate

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Introduction

Hypoglycemia is a common metabolic condition during development and may lead to severe neurological defects in human infants. However, the effects of hypoglycemia on the developing brain are still incompletely understood (Comblath et al. 2000). The brain derives more than 99 % per cent of its energy from the oxidation of glucose. The developing brain is thought to have an ability to utilize alterative energy substrates which propensity may protect it during hypoglycemia (Nehlig and Pereira de Vasconcelos

1993; Vannucci and Vannucci 2001). On the other hand, poor reserves of high-energy phosphates and high metabolic rate may predispose the developing brain to hypoglycemic injury (McGowan and Perlman 2006; Burns et al. 2008). The hippocampus is the region most sensitive to oxygen and glucose lack. Most excitatory synaptic transmission in the hippocampus is mediated by the ionotropic glutamate receptors of the 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and *N*-methyl-D-aspartate (NMDA) type. Energy deprivation leads to neuronal cell death, primarily caused by excitotoxicity due to excessive glutamate release (Szatkowski and Attwell 1994; Lee et al. 1999). In addition to this mechanism, a reversal of glutamate uptake by astrocytes to release contributes to cell damage (Jabaudon et al. 2000; Rossi et al. 2000). A transient hypoglycemia has been shown to potentiate evoked NMDA receptor-dependent synaptic responses and even more so those governed by AMPA (Quintana et al. 2006).

Taurine (2-aminoethanesulfonic acid) is a simple sulfur-containing inhibitory amino acid ubiquitous in virtually all animal cells. It is present at high concentrations in the brain, especially during ontogenic development its concentration exceeds the concentration of the main excitatory transmitter glutamate (Saransaari and Oja 2000). It increases membrane chloride conductance, causing hyperpolarization and inhibiting neuronal firing (Oja et al. 1977, 1990). In this manner it could protect neural cells against the toxicity of excitatory amino acids in the hippocampus (French et al. 1986). Cell-damaging conditions, including hypoglycemia, increase the release of excitatory amino acid neurotransmitters in the hippocampus but also the release of taurine (Saransaari and Oja 1997a, 1998). Furthermore, the activation of ionotropic glutamate receptors enhances taurine release (Saransaari and Oja 1997b) and metabotropic glutamate receptors also participate in the regulation of taurine release in both developing and adult hippocampus (Saransaari and Oja 1999). However, no data are available on the regulation of taurine release by glutamate receptors in hypoglycemia. Our primary assumption was that glutamate receptors could preserve their ability to affect taurine release in the absence of adequate supply of glucose and taurine could also under these conditions protect neural cells from injury. In the present study we therefore examined how taurine release is influenced by glutamate receptor agonists and antagonists and whether taurine is released from the hippocampus in such amounts that it could counteract the excitotoxicity caused by liberation of excitatory amino acids in glucose-free media in the developing and adult hippocampus. In many experiments the slices were also exposed to depolarizing K^+ concentrations to investigate whether the simultaneous activation of glutamate receptors could further enhance taurine release.

Materials and methods

Materials

Developing (7 days old) and young adult (3 months old) NMRI mice of both sexes were used in the experiments. All efforts were made to minimize both suffering and number of the animals used. The experiments conformed to the European Community Directive (86/609/EEC) for ethical use of experimental animals and were approved by the Committee of Tampere University for animal experiments. [^{3}H]Taurine (specific radioactivity 1.15 PBq/mol) was obtained from Amersham International, Bristol, UK. (2R,4R)-4-Aminopyrrolidine-2,4-dicarboxylate (2R,4R-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), 2S-2-ethylglutamate (EGLU), L(+)-2-amino-4-phosphonobutyrate (L-AP4), *O*-phospho-L-serine (L-SOP), dizocilpine [(5S, 10R)-(+)-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), and (1±)-1-aminocyclopentane-*trans*-1,3-dicarboxylate (t-ACPD) were from Tocris Cookson (Bristol, UK). Other reagents and drugs were from common commercial sources.

Release experiments

Coronal slices 0.4 mm thick weighing 15–20 mg were manually prepared from the mouse hippocampus 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 30 min under agitation. The standard Krebs–Ringer–Hepes 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 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). Hypoglycemic conditions were induced by omitting glucose from the superfusion media. Potassium stimulation was applied from 30 to 50 min with 50 mM K^+. In out experimental setup this K^+ concentration has yielded the best and most reproducible responses in GABA and taurine release (Kontro and Oja 1987). The glutamate receptor agonists were added to medium at the onset of superfusions and their antagonists either at the onset or at 30 min, as

explained in the figure and table legends. The superfusion medium was pooled during the first 20 min whereafter 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, and centrifuged, and the clear supernatants were used for scintillation counting. The effluent samples were subjected to the same analyses.

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). During superfusion the release of labeled taurine initially originates from the extracellular spaces in slices. This source is gradually exhausted during the first 20 min and the release subsequently occurs from the intracellular pools. The efflux rate constants of taurine for the time intervals of 20–30 min (k_1, initial release phase) and 34–50 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. When the medium was changed to the high-K^+ medium (50 mM) at 30 min then third rate constants (k_3) were also calculated for the time interval of 34–50 min.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to compare the effects of glutamate receptor agonists and their antagonists. When ANOVA showed a significant difference, the post hoc Bonferroni test was applied to demonstrate the differences. They were considered significant when the calculated p values were less than 0.05 or 0.01.

Results

There were no significant differences in the results between male and female mice. Glucose-free media markedly enhanced the release rate of [^{3}H]taurine in 7-day-old mice. In adult 3-month-old mice the enhancement was also significant but considerably less in magnitude (Fig. 1). In the absence of glucose, K^+ stimulation was able to enhance the release in both age groups, the effect being relatively greater in adults (cf. constants k_2 and k_3 in Tables 1, 3).

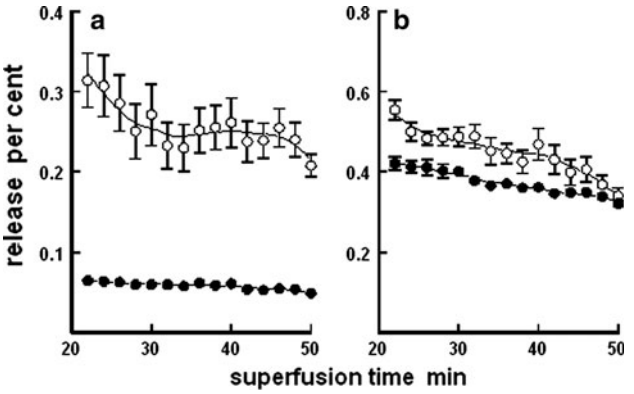

Fig. 1 Taurine release from hippocampal slices from 7-day-old (**a**) and 3-month-old (**b**) mice. Standard incubation conditions with glucose (*filled circle*) and incubation without glucose (*open circle*). Release of labeled taurine in percent of the total radioactive taurine into 2-min superfusion fractions. Mean values ± SEM (if it exceeds the size of symbols) are shown. Number of independent experiments 11. Please, note the twofold difference in the scales of y axis

Adult mice

When added to the superfusion medium at the onset, glutamate (0.1 mM) had no significant effect on taurine release in adult mice (Table 1). All ionotropic glutamate receptor agonists enhanced the initial taurine release (k_1) ($F = 16.453$, $df = 3$, $p = 0$). Kainate and AMPA (both 0.1 mM) potentiated the initial (k_1) release in adults, but did not affect the K^+-stimulated release (k_3). The later phase of unstimulated release (k_2) was also enhanced ($F = 5.7701$, $df = 3$, $p = 0.0032$). Kainate and NMDA were effective but AMPA did not cause a statistically significant effect. The enhancement of NMDA on the release was effectively attenuated by its antagonist MK-801 (Fig. 2a). NMDA did not affect the release evoked by K^+ stimulation (Table 1). The antagonist CNQX (0.1 mM) blocked the kainate stimulation (Fig. 2b). The AMPA antagonist NBQX (0.1 mM) did not reduce the AMPA stimulation. On the contrary, it even tended to further enhance the release in the presence of AMPA, the K^+-stimulated release in particular (Table 1). NBQX was also markedly effective in the absence of AMPA, whereas the other antagonists alone were not effective.

The metabotropic glutamate receptor agonists and antagonists were also added at the onset of superfusions. In adult mice, the group I agonists t-ACPD and DHPG and the group II agonist 2R,4R-APDC (all 0.1 mM) were not effective (Table 2). The group III agonist L-AP4 (0.1 mM) significantly enhanced the release (both k_1 and k_2). The effects of L-AP4 were blocked by its antagonist CPPG (0.1 mM) (Fig. 3a), which alone was not effective (Table 2). However, the other group III agonist L-SOP (0.1 mM) was not effective. The K^+-stimulated release (k_3) was now not affected by any of the above agonists.

Table 1 Effects of the agonists and antagonists of ionotropic glutamate receptors on taurine release from hippocampal slices from 3-month-old mice in glucose-free media

Concentration (0.1 mM)	Eefflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM		
	k_1	k_2	k_3 (50 mM K^+)
Basal (control)	2.36 ± 0.11 (20)	1.93 ± 0.18 (11)	2.92 ± 0.21 (10)
Glutamate	2.78 ± 0.10 (8)	2.63 ± 0.18 (4)	2.74 ± 0.20 (4)
NMDA	2.87 ± 0.12 (8)*	3.11 ± 0.30 (6)**	2.93 ± 0.21 (6)
NMDA + MK-801	1.98 ± 0.12 (6)[a]	1.50 ± 0.15 (4)	2.78 ± 0.07 (4)
Kainate	3.00 ± 0.06 (12)**	2.96 ± 0.30 (7)**	3.06 ± 0.25 (4)
Kainate + CNQX	2.31 ± 0.19 (8)[b]	1.88 ± 0.12 (4)	2.25 ± 0.25 (4)
AMPA	3.36 ± 0.06 (7)**	2.37 ± 0.08 (8)	2.83 ± 0.22 (4)
AMPA + NBQX	3.36 ± 0.21 (8)**	2.63 ± 0.21 (4)	4.14 ± 0.41 (4)**
MK-801	2.31 ± 0.17 (8)	2.32 ± 0.13 (4)	2.81 ± 0.10 (4)
CNQX	2.23 ± 0.21 (7)	2.38 ± 0.16 (4)	2.74 ± 0.32 (4)
NBQX	3.13 ± 0.19 (8)**	2.56 ± 0.21 (4)	4.11 ± 0.46 (4)**

The slices were first preloaded for 30 min with 10 μM [^{3}H]taurine in Krebs–Ringer–Hepes–glucose medium, pH 7.4, and then superfused with the same medium without glucose for 50 min. The ionotropic 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 in parentheses

NMDA *N*-methyl-D-aspartate, *MK-801* dizocilpine, (5S, 10R)-(+)-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

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] $p < 0.01$

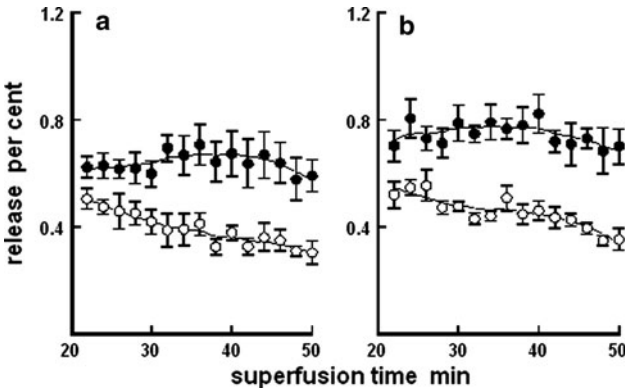

Fig. 2 Taurine release from hippocampal slices from 3-month-old mice in glucose-free media. **a** Release into 2-min superfusion fractions in the presence of 0.1 mM *N*-methyl-D-aspartate (NMDA) (*filled circle*) and in the presence of 0.1 mM NMDA together with 0.1 mM dizocilpine [(5S, 10R)-(+)-methyl-10,11-dihydro-5*H*-dibenzo(a,d)cyclohepten-5,10-amine, MK-801] (*open circle*). **b** Release into 2-min superfusion fractions in the presence of 0.1 mM kainate (*filled circle*) and in the presence of 0.1 mM kainate together with 0.1 mM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (*open circle*). Mean values ± SEM are shown. Number of independent experiments 6–8 (NMDA and MK-801) and 4–12 (kainate and CNQX)

Developing mice

In developing mice, 0.1 mM glutamate was not effective (Table 3). The ionotropic glutamate agonists were enhancing ($F = 16.7782$, $df = 3$, $p = 0$). Kainate and AMPA, when added at the onset, potentiated only the initial (k_1) taurine release. The later release phase was also enhanced ($F = 6.203$, $df = 3$, $p = 0.0048$), but again not by AMPA. NMDA and kainate enhanced the release during all phases, the K^+-stimulated release included (Table 3). The antagonist MK-801 attenuated the initial NMDA stimulation but did not affect the later phases of NMDA-stimulated release. CNQX attenuated the effect of kainate, but again at this age the AMPA antagonist NBQX further enhanced the AMPA effect (Table 3). NBQX was also effective without AMPA and MK-801 and CNQX alone were without significant effect.

In developing mice, the metabotropic glutamate receptor group I agonists t-ACPD and DHPG (both 0.1 mM) were likewise not effective on the unstimulated release (k_1 and k_2), but t-ACPD enhanced the K^+-stimulated release (k_3) (Table 4). The group II agonist 2R,4R-APDC (0.1 mM) initially slightly enhanced the initial release (k_1), being not effective during the later release phases. This initial effect was further enhanced by the antagonist EGLU (0.1 mM). EGLU alone also enhanced the release (Table 4). At this age the group III agonist L-AP4 was not effective but L-SOP diminished the initial release (k_1) which effect was reversed by the antagonist CPPG (Fig. 3b). CPPG was not effective when tested alone. The K^+-stimulated release (k_3) was not affected by the above effectors with the only exception of t-ACPD which slightly tended to potentiate

Table 2 Effects of agonists and antagonists of metabotropic glutamate receptors on taurine release from hippocampal slices from 3-month-old mice in glucose-free media

Concentration (0.1 mM)	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM		
	k_1	k_2	k_3 (50 mM K^+)
Basal (control)	2.36 ± 0.11 (20)	1.93 ± 0.18 (11)	2.92 ± 0.21 (10)
t-ACPD	2.16 ± 0.13 (15)	1.61 ± 0.13 (7)	2.33 ± 0.34 (4)
DHPG	2.58 ± 0.30 (16)	2.24 ± 0.25 (12)	2.91 ± 0.29 (7)
2R, 4R-APDC	2.67 ± 0.22 (8)	1.97 ± 0.12 (4)	2.26 ± 0.08 (4)
L-AP4	3.11 ± 0.22 (7)**	2.71 ± 0.24 (4)*	3.43 ± 0.29 (4)
L-AP4 + CPPG	2.40 ± 0.16[b] (7)	1.48 ± 013[a] (4)	2.67 ± 0.15 (4)
L-SOP	2.38 ± 0.21 (7)	2.02 ± 0.13 (4)	3.09 ± 0.29 (4)
CPPG	2.52 ± 0.25 (8)	2.09 ± 0.21 (4)	3.29 ± 0.32 (4)

The slices were first preloaded for 30 min with 10 μM [^{3}H]taurine in Krebs–Ringer–Hepes–glucose medium, pH 7.4, and then superfused with the same medium without glucose for 50 min. The metabotropic glutamate receptor agonists and antagonists were present from the beginning of superfusions. The results are efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM. Number of independent experiments in parentheses

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

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] $p < 0.01$

the release (Table 4). This effect was blocked by the antagonist AIDA added to the superfusion medium at the onset of K^+ stimulation at 30 min.

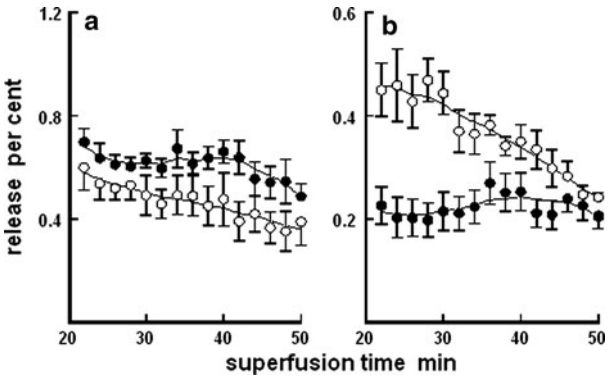

Fig. 3 Taurine release from hippocampal slices in glucose-free media. **a** Release from slices from 3-month-old mice into 2-min superfusion fractions in the presence of 0.1 mM L(+)-2-amino-4-phosphonobutyrate (L-AP4) (*filled circle*) and in the presence of 0.1 mM L-AP4 together with 0.1 mM (RS)-2-cyclopropyl-4-phosphonophenylglycine) CPPG) (*open circle*). **b** Release from slices from 7-day-old mice into 2-min superfusion fractions in the presence of 0.1 mM *O*-phospho-L-serine (L-SOP) (*filled circle*) and in the presence of 0.1 mM L-SOP together with 0.1 mM CPPG (*open circle*). Mean values ± SEM are shown. Number of independent experiments 4–7. Please, note the twofold difference in the scales of *y* axis

Discussion

General properties of release

Glucose-free media induced a marked increase in taurine release, in particular in the developing hippocampus. The enhancement could be due to several mechanisms, including Ca^{2+}-dependent exocytosis, Ca^{2+}-independent release via reversal of carrier-mediated uptake, indiscriminate opening of ion channels which allow the passage of taurine molecules or leakage through damaged plasma membranes. This last alternative seems not likely since lactate dehydrogenase, a common marker of cell plasma membrane and lysis of neural cells (Pellegrini-Giampietro et al. 1990; Cherici et al. 1991), does not leak out from slices into medium under our experimental conditions (Saransaari and Oja 1997a). Depolarization by K^+-stimulation was now able to further potentiate taurine release at both ages under hypoglycemia. This may signify release via exocytosis which is also preserved in hippocampal slices when they are exposed to even more drastic cell-damaging conditions such as ischemia (the absence of glucose and oxygen atmosphere) (Saransaari and Oja 1997a, 1998). In developing mice taurine release in the absence of glucose is about the same as in the absence of glucose and in the presence of nitrogen atmosphere, whereas in adults these ischemic conditions have been even more efficient (Saransaari and Oja 1998). In adults, it could be assumed that glutamate released may thus not fully saturate glutamate receptors. However, only in developing mice ionotropic glutamate receptor agonists were able to enhance further taurine release evoked by K^+ stimulation, which is at variance with the above assumption.

Table 3 Effects of the agonists and antagonists of ionotropic glutamate receptors on taurine release from hippocampal slices from 7-day-old mice in glucose-free media

Concentration (0.1 mM)	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM		
	k_1	k_2	k_3 (50 mM K^+)
Basal (control)	1.34 ± 0.08 (31)	1.20 ± 0.11 (11)	1.90 ± 0.10 (12)
Glutamate	1.66 ± 0.11 (7)	1.42 ± 0.07 (4)	1.95 ± 0.07 (4)
NMDA	2.19 ± 0.11 (8)**	1.65 ± 0.06 (4)*	2.35 ± 0.18 (4)*
NMDA + MK-801	1.67 ± 0.17 (8)[b]	1.68 ± 0.17 (8)**	2.59 ± 0.15 (4)*
Kainate	2.04 ± 0.15 (12)**	1.35 ± 0.03 (7)	2.32 ± 0.18 (4)*
Kainate + CNQX	1.99 ± 0.15 (16)	1.20 ± 0.13 (8)	1.75 ± 0.11 (4)[b]
AMPA	2.29 ± 0.12 (25)**	1.53 ± 0.04 (8)	2.23 ± 0.10 (4)
AMPA + NBQX	2.71 ± 0.21 (13)**	1.89 ± 0.13 (9)	3.10 ± 0.11 (8)**
MK-801	1.11 ± 0.21 (8)	1.09 ± 0.15 (4)	2.13 ± 0.15 (4)
CNQX	1.07 ± 0.07 (8)	1.00 ± 0.12 (4)	2.05 ± 0.19 (4)
NBQX	2.65 ± 0.11 (11)**	1.75 ± 0.18 (4)**	3.02 ± 0.08 (4)**

The slices were first preloaded for 30 min with 10 μM [^{3}H]taurine in Krebs–Ringer–Hepes–glucose medium, pH 7.4, and then superfused with the same medium without glucose for 50 min. The ionotropic 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 in parentheses

NMDA N-methyl-D-aspartate, *MK-801* dizocilpine, (5S, 10R)-(+)-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

Significance of differences from the control: * $p < 0.05$; ** $p < 0.01$

[b] Significance of differences between an agonist and the corresponding antagonist ($p < 0.01$)

Table 4 Effects of agonists and antagonists of metabotropic glutamate receptors on taurine release from hippocampal slices from 7-day-old mice in glucose-free media

Concentration (0.1 mM)	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM		
	k_1	k_2	k_3 (50 mM K^+)
Basal (control)	1.34 ± 0.08 (31)	1.20 ± 0.11 (11)	1.90 ± 0.10 (12)
t-ACPD	1.60 ± 0.13 (24)	1.17 ± 0.02 (4)	2.42 ± 0.20* (12)
t-ACPD + AIDA	–	–	2.02 ± 0.14 (8)
DHPG	1.22 ± 0.13 (7)	1.02 ± 0.07 (4)	1.67 ± 0.18 (4)
2R, 4R-APDC	1.77 ± 0.17 (16)*	1.07 ± 0.15 (4)	2.15 ± 0.08 (4)
2R, 4R-APDC + EGLU	1.97 ± 0.19* (4)	1.36 ± 0.13 (4)	2.06 ± 0.06 (4)
L-AP4	1.49 ± 0.12 (8)	1.06 ± 0.11 (4)	2.14 ± 0.08 (4)
L-SOP	0.91 ± 0.10 (7)**	1.00 ± 0.12 (4)	1.85 ± 0.17 (4)
L-SOP + CPPG	1.74 ± 0.14 (4)[a]	1.35 ± 0.09 (4)	2.25 ± 0.10 (4)
EGLU	1.83 ± 0.06 (4)**	1.45 ± 0.12 (4)	–
CPPG	1.57 ± 0.13 (8)	1.24 ± 0.10 (4)	2.18 ± 0.11 (4)

The slices were first preloaded for 30 min with 10 μM [^{3}H]taurine in Krebs–Ringer–Hepes–glucose medium, pH 7.4, and then superfused with the same medium without glucose for 50 min. The metabotropic glutamate receptor agonists and most of the antagonists were present from the beginning of superfusions. Only AIDA was added at 30 min. The results are efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM. Number of independent experiments in parentheses

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

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

Both neurons and glial cells have been shown to contain taurine (Oja and Saransaari 2007) and thus only a part of the released taurine may originate from neurons. K^+ stimulation of taurine release has also been shown to be associated with cell swelling (Schousboe et al. 1990). Brain slices swell under anaerobic conditions (Laakso and Oja

1976) and intracellular swelling activates stretch-sensitive ion channels and is accompanied by the release of both inorganic and organic osmolytes, including taurine (Pasantes-Morales et al. 1993). This swelling-induced increase in taurine release has been shown to be a diffusional process without any carrier involvement (Sanchez-Olea et al. 1993). Non-saturable diffusion of taurine is indeed greatly enhanced under ischemic conditions (Saransaari and Oja 1996).

Taurine molecules released from the cells must first traverse through the extracellular spaces to reach their destination of superfusion medium. During this process some of them are recaptured into cells by uptake in spite of the continuous flow of medium. Indeed, there obtains a significant positive correlation between the influx and efflux of taurine in brain slices (Oja and Saransaari 1994). Taurine uptake is an energy-requiring process (Lähdesmäki and Oja 1973). The energy supply for a concentrative uptake is compromised in hypoglycemia and the uptake mechanisms are inhibited. The enhanced release of excitatory amino acids under hypoxic and ischemic conditions has likewise held to result from reversed operation of cell membrane carriers (Szatkowski and Attwell 1994). The concentration of taurine in the developing hippocampus is markedly greater than in the adult hippocampus (Oja and Saransaari 2009). Taurine uptake under normoglycemic conditions is also markedly more efficient in developing than in adult mice (Oja and Kontro 1983). The high intracellular taurine content and the impairment of the efficient uptake mechanisms are thus likely to underlie at least partly the greater enhancement of the apparent taurine release in the developing hippocampus in the absence of glucose.

Effects of ionotropic glutamate receptors

In spite of the larger enhancement of taurine release in slices from developing than adult mice, the responses to glutamate receptors agonists and antagonists were preserved in both. The hippocampal formation is functionally a feed-forward synaptic chain, in which all three synaptic links in the pathway are excitatory and use glutamate as transmitter (Frotscher 1988). Presynaptic ionotropic glutamate receptors have been shown to regulate neurotransmitter release in the adult hippocampus (Pittaluga and Raiteri 1992; Janáky et al. 1993; Smirnova et al. 1993). Of the ionotropic receptors, NMDA receptor activation has been assumed to play a central role in hypoglycemia-induced glutamate release (Ichord et al. 2001). All three classes of ionotropic glutamate receptors have enhanced taurine release in the developing and adult hippocampus under standard oxygenated conditions in the presence of glucose (Saransaari and Oja 1997a). In keeping with these findings all of them were still able to enhance taurine release in the absence of glucose when taurine release is much greater than in control normoglycemic conditions. Activation of NMDA receptors allows Ca^{2+} to enter the cells. NO synthase is a Ca^{2+}-dependent enzyme (Bredt et al. 1992), being activated in the presence of Ca^{2+} and then producing NO. NO stimulates soluble guanylate cyclase and in this manner foments the production of 3′,5′-cyclic guanosine monophosphate (Schuman and Madison 1994), which enhances taurine release (Saransaari and Oja 2002).

NMDA has most often been the most effective in stimulating taurine release (Magnusson et al. 1991; Saransaari and Oja 1997b) but now NMDA and kainate were almost equipotent. Their efficacy was preserved even in the developing hippocampus in spite of the large increase in taurine release in the absence of glucose. In keeping with these results, hippocampal slices from young rats have been shown to preserve their viability and maintain the ATP levels better than slices from adult rats (Kass and Lipton 1989). For instance, taurine has been shown to attenuate markedly the ischemia-induced reduction in ATP level in the rat brain (Sun et al. 2011).

In adult mice the enhancements by NMDA and kainate were clearly receptor-mediated since they were blocked by their specific antagonists MK-801 and CNQX, respectively. The enhancements signify that endogenous glutamate released does not saturate the receptors. On the other hand, the above antagonists alone did not significantly affect the release, indicating that basal release in glucose-free media is independent of the action of endogenous glutamate on these receptors. An anomalous finding, hard to explain, was that the AMPA receptor antagonist NBQX, which also acts at kainate receptors (Sheardown 1993), did not abolish the AMPA effect. On the contrary, it even enhanced now the K^+ stimulation in the presence of AMPA and enhanced taurine release in the absence of its agonist AMPA in both developing and adult hippocampus. According to the current opinion AMPA receptors are responsible for the bulk of fast excitatory synaptic transmission and function in synergy with NMDA receptors, governing downstream NMDA receptor activation (Mayer 2005). However, the recently discovered transmembrane AMPA receptor regulatory proteins have challenged this straightforward concept (Rao and Finkbeiner 2007, Milstein and Nicoll 2008.) For example, they can switch quinoxalidine AMPA antagonists into partial agonists (Menuz et al. 2007) and hence enhance taurine release.

Effects of metabotropic glutamate receptors

It is a little difficult to draw definite inferences of the involvement of metabotropic glutamate receptors

(mGluRs) on taurine release, since the agonists and their agonists are not strictly specific for their receptors and there obtains heterogeneity among mGluRs. They also have different effects on the neuronal recovery in rat hippocampal slices after hypoglycemia (Opitz et al. 1995). All classes of mRNAs of different presynaptic metabotropic glutamate receptors have been demonstrated to be present in the hippocampus (Abe et al. 1992; Shigemoto et al. 1992; Nakanishi 1994). Presynaptic metabotropic receptors have been shown to regulate the release of excitatory amino acid neurotransmitters in the cerebral cortex and striatum (Herrero et al. 1994; East et al. 1995; Lombardi et al. 1996), and the release of GABA in hippocampal and brain stem slices (Saransaari and Oja 2001, 2005) and in the periaqueductal grey (de Novellis et al. 2003), facilitation or inhibition depending on which mGluR is activated. They also modify taurine release in the developing and adult hippocampus under standard incubation conditions in the presence of glucose (Saransaari and Oja 1999). Their efficacy has been weaker, however, than that of ionotropic glutamate receptors. So was the case also now in glucose-free media. Also in the brain stem ionotropic glutamate receptors have shown to be more effective under both normoxic and ischemic conditions (Saransaari and Oja 2010). The present results on the effects of metabotropic glutamate receptors in glucose-free media are likewise inconclusive.

The metabotropic receptor group I agonist t-ACPD enhanced only the K^+ stimulation of taurine release in developing mice. The effect seems to be receptor-mediated because it was blocked by the agonist AIDA. The other group I receptor agonist DHPG was not effective at all. In keeping with these results, the group I receptors have been only marginally effective in the presence of glucose in the developing hippocampus, but not in the adult (Saransaari and Oja 1999). The mGlu1 receptor mRNA is expressed in the hippocampus (Shigemoto et al. 1992), but the isoform composition of mGlu1 subtypes is altered during postnatal development (Minakami et al. 1995) which change may underlie the loss of its efficacy on taurine release. The non-selective group I/group II metabotropic glutamate receptor antagonist (RS)-α-methyl-4-carboxyphenylglycine has also been shown to protect hippocampal CA1 neurons in rats against hypoglycemia (Opitz et al. 1994).

The group II and III mGluRs generally reduce synaptic activation, acting as inhibitory autoreceptors (Nicoletti et al. 1996; Sánchez-Prieto et al. 1996). For instance, mGluR2 receptors tonically inhibit glutamate release from corticostriatal terminals (Cozzi et al. 1997). The group II agonist 2R,4R-APDC was now ineffective in the adult hippocampus and only enhanced the initial taurine release in the developing hippocampus. The group II mGluRs thus do not essentially participate in the regulation of taurine release. In the developing hippocampus the group III receptor agonist L-AP4 was not effective, but in the adult hippocampus it significantly enhanced taurine release which effect was blocked by its agonist CPPG. The effect seems thus to be receptor-mediated. Also the release of glycine from the mouse brain stem has been enhanced by group III agonists in a receptor-mediated manner (Saransaari and Oja 2009). On the other hand, the other group III agonist L-SOP was not active in the adult hippocampus but markedly inhibited the initial taurine release in the developing hippocampus. The effect was blocked by the antagonist CPPG. The results demonstrate marked differences in the responses to mGluRs belonging to the groups II and III.

Conclusions

Taurine release from hippocampal slices is affected by the ionotropic glutamate receptors under hypoglycemic conditions but the metabotropic glutamate receptors play minor roles in the regulation of taurine release. The release of the inhibitory neuromodulator taurine may maintain homeostasis in the brain, counteracting any excitotoxic effects of glutamate possibly released in excess. It has been suggested that taurine reduces glutamate excitotoxicity through both the enhancement of mitochondrial functions and the regulation intracellular Ca^{2+} homeostasis (El Idrissi 2008). Enhanced taurine release could be particularly important in the developing hippocampus. GABA cannot protect against excitotoxicity in the developing hippocampus, since during early development GABA is rather excitatory than inhibitory (Ben-Ari 2002). The rationale is that the activation of excitatory amino acid receptors causes at the same time an increase in taurine release.

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Conflicts of interest The authors declare that they have no conflict of interest.

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