Amino Acids (2003) 24: 213–221
DOI 10.1007/s00726-002-0310-z

Amino
Acids
Printed in Austria

Characterization of N-methyl-D-aspartate-evoked taurine release in the developing and adult mouse hippocampus

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Received February 7, 2002
Accepted March 6, 2002
Published online August 20, 2002;

Summary. Taurine is an inhibitory amino acid acting as an osmoregulator and neuroromodulator in the brain, with neuroprotective properties. The ionotropic glutamate receptor agonist N-methyl-D-aspartate (NMDA) greatly potentiates taurine release from brain preparations in both normal and ischemic conditions, the effect being particularly marked in the developing hippocampus. We now characterized the regulation of NMDA-stimulated taurine release from hippocampal slices from adult (3-month-old) and developing (7-day-old) mouse using a superfusion system. The NMDA-stimulated taurine release was receptor-mediated in both adult and developing mouse hippocampus. In adults, only NO-generating compounds, sodium nitroprusside, S-nitroso-N-acetylpenicillamine and hydroxylamine reduced the release, as did also NO synthase inhibitors, 7-nitroindazole and nitroarginine, indicating that the release is mediated by the NO/cGMP pathway. On the other hand, the regulation of the NMDA-evoked taurine release proved to be somewhat complex in the immature hippocampus. It was not affected by the NOergic compounds, but enhanced by the protein kinase C activator 4β-phorbol 12-myristate 13-acetate and adenosine receptor A_1 agonists, N^6-cyclohexyladenosine and R(−)N^6-(2-phenylisopropyl)adenosine in a receptor-mediated manner. The activation of both ionotropic 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors and metabotropic glutamate group I receptors also enhanced the evoked release. The NMDA-receptor-stimulated taurine release could be a part of the neuroprotective properties of taurine, being important particularly under cell-damaging conditions in the developing hippocampus and hence preventing excitotoxicity.

Keywords: Taurine release – N-Methyl-D-aspartate receptors – Hippocampal slices – Adult – Developing mouse

Abbreviations: AIDA: RS-1-aminoindan-1,5-dicarbohydroxylate; AMPA: 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; cGMP: 3′,5′-cyclic guanosine monophosphate; CHA: N^6-cyclohexyladenosine; DCGIV:(2S,2R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine; DHPG: (S)-3,5-dihydroxyphenylglycine; DPCPX: 8-cyclopentyl-1,3-dipropylxanthine; H-7: 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride; HA: hydroxylamine; L-AP3: L(+)-2-amino-3-phosphonopropionate; L-AP4: L(+)-2-amino-4-phosphonobutyrate; L-NAME: N^G-nitro-L-arginine methyl ester; L-NNA: N^G-nitro-L-arginine; L-SOP: O-phospho-L-serine; NBQX: 2,3-dioxo-6-nitro-1,2,3,4-tetra-hydrobenzo[f] quinoxaline-7-sulfonamide; NBTI: S-(4-nitrobenzyl)-6-thioinosine; 7-NINA: 7-nitroindazole; NMDA: N-methyl-D-aspartate; PMA: 4β-phorbol 12-myristate 13-acetate; R-PIA: R(−)N^6(2-phenylisopropyl)adenosine; SNAP: S-nitroso-N-acetylpenicillamine; SNP: sodium nitroprusside; trans-ACPD: (1±)-1-aminocyclopentane-trans-1,3-dicarboxylate.

Introduction

Taurine (2-aminoethanesulfonic acid) is a most abundant free amino acid in the central nervous system, its concentration even exceeding that of glutamate during ontogenic development in many mammals (Oja and Kontro, 1983a). This inhibitory amino acid has been thought to function as a regulator of neuronal activity, particularly in the developing brain, being also involved in osmoregulation and cell volume adjustments (Huxtable, 1992; Oja and Saransaari, 1996). Moreover, taurine has been shown to protect neural cells from excitotoxicity induced by excitatory amino acids in the hippocampus (French et al., 1986) and cerebellum (Trenkner, 1990), to prevent harmful metabolic sequences evoked by ischemia or hypoxia (Schurr et al., 1987), and to alleviate symptoms in epilepsy (Oja and Kontro, 1983b). Taurine protects cerebellar granule cells exposed to kainate without affecting the production of reactive oxygen species (Boldyrev et al., 1999). An endogenous taurine-containing dipeptide, γ-L-glutamyltaurine, also efficiently attenuates glutamate-agonist-evoked calcium fluxes in neurons (Varga et al.,

1992). Taurine-containing neurons enriched by taurine are fairly resistant to cerebral ischemia induced by four-vessel occlusion (Wu et al., 1994).

Various cell-damaging conditions induce a substantial release of taurine in the hippocampus, ischemia being here particularly effective (Saransaari and Oja, 1997a; 1998; 2000b). Ischemic conditions are known to cause a massive increase in the release of excitatory amino acids, which then activate glutamate receptors, particularly those of the N-methyl-D-aspartate (NMDA) class. The associated ion channels open, elevating intracellular Ca^{2+} and potentiating the NMDA-receptor-gated currents (Szatkowski and Attwell, 1994; Nishizawa, 2001). The abnormal function of NMDA receptors has been implicated in a variety of psychiatric and neurological disorders, including schizophrenia, epileptic seizures and neuronal cell death (Ozawa et al., 1998; Cull-Candy et al., 2000).

Agonists of the glutamate receptors, especially those of the NMDA class, also greatly stimulate taurine release in both normoxia and ischemia, the potentiation of release being particularly marked in the developing hippocampus (Saransaari and Oja, 1997b; Oja and Saransaari, 2000). The NMDA-stimulated taurine release could thus contribute to mechanisms protective against excitotoxicity, being especially important the immature brain, which is susceptible to excitotoxic neuronal damage (Cook and Crutcher, 1986). Little is known, however, of the properties and regulation of this release. We now therefore characterized the NMDA-stimulated taurine release from the adult (3-month-old) and developing (7-day-old) mouse hippocampus using a superfusion system, with an emphasis on the effects of glutamate-receptor agonists, NO-generating compounds and adenosine.

Materials and methods

Material

NMRI mice of both sexes aged 7 days and 3 months (adults) were used throughout. [1,2-^{3}H]Taurine (specific activity 1.07 PBq/mol) was obtained from Amersham International, Bristol, UK, and all glutamate receptor agonists and antagonists, NO-generating compounds, NO synthase inhibitors, protein kinase C activators and inhibitors and adenosine receptor effectors from Tocris Cookson, Bristol, UK and Sigma, St. Louis, MO.

Release experiments

Slices 0.4 mm thick weighing 15–20 mg were prepared with a Stadie-Riggs tissue slicer from the hippocampi of mice and used immediately in efflux experiments. The slices were preloaded for 30 min with 10 μM (50 MBq/l) [^{3}H]taurine in preoxygenated Krebs-Ringer-Hepes-glucose medium under O_2 and superfused with the same medium as in Kontro and Oja (1987), modified in some experiments as indicated. The medium was pooled during the first 20 min of superfusion, whereafter 2-min fractions (0.5 ml) were collected. At 30 min the medium was often changed to modified medium. After superfusion the slices were weighed, homogenized in ice-cold 5% (w/v) trichloracetic acid solution and centrifuged, and the clear supernatants used for scintillation counting. The effluent samples were subjected to the same analyses. In Na^+-free media NaCl was substituted by equimolar choline chloride, and in Mg^{2+}-free media $MgCl_2$ by equimolar choline chloride.

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 superfusate fractions collected (Kontro and Oja, 1987). The efflux rate constants of taurine for the time interval of 34 to 50 min (k_2) were computed as negative slopes for the regression lines of the logarithm of radioactivity remaining in the slices vs. superfusion time.

Statistical calculations

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

Results

When the hippocampal slices were already superfused with 0.1 mM NMDA from the outset onwards, the release of preloaded taurine was greatly enhanced in 7-day-old mice (Fig. 1A) and also in 3-month-olds (Fig. 2). When 0.1 nM NMDA was added to the superfusion medium at 30 min, the release was enhanced by about 150% and 900% in the adult and developing hippocampus, respectively (see Fig. 4). The NMDA-evoked release was reduced by the specific antagonist dizocipline at both ages (Table 1). The efflux rate constants were not significantly different when the experiments were carried out in Mg^{2+}-free media (data not shown). Glutamate receptor agonists affected the NMDA-evoked taurine release only in 7-day-old mice (Table 1). Of the ionotropic glutamate receptor agonists, only 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) at 0.1 mM stimulated the NMDA-evoked release, which effect was abolished by the antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (0.1 mM) (Table 1). Of the metabotropic agonists, the group I agonists, (1±)-1-aminocyclopentane-trans-1,3-dicarboxylate (trans-ACPD) and (S)-3,5-dihydroxyphenylglycine (DHPG) (both 0.1 mM) enhanced the release (Table 1). The

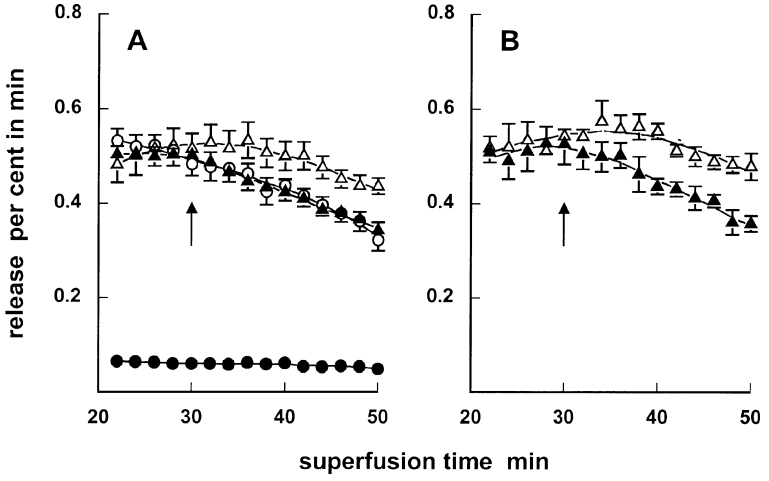

Fig. 1. **A** Time courses of the basal (-●-) and 0.1 mM NMDA-evoked taurine release (-○-) from hippocampal slices from 7-day-old mice and modification of the NMDA-evoked release in the presence of 0.1 mM (S)-3,5-dihydroxyphenylglycine (DHPG) (-△-) and 0.1 mM DHPG together with 0.1 mM (RS)-1-aminoindan-1,5-dicarboxylate (AIDA) (-▲-). **B** Time course of the NMDA-evoked release in the presence of 0.1 mM 4β-phorphol 12-myristate 13-acetate (PMA) (-△-) and the effect of 0.1 mM 1-(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride (H-7) (-▲-) on this release. NMDA, DHPG and PMA were present from the beginning of superfusion, while AIDA and H-7 were added at 30 min, as shown by the arrows. The results are mean values ± SEM of 4–8 independent experiments

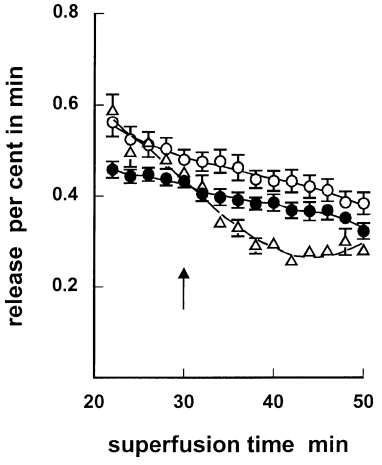

Fig. 2. Time courses of basal (-●-) and 0.1 mM NMDA-evoked taurine release (-○-) from mouse hippocampal slices from 3-month-old mice and modification of the NMDA-evoked release in the presence of 1.0 mM S-nitroso-N-acetylpenicillamine (SNAP) (-△-). NMDA (0.1 mM) was applied from the beginning of the superfusion and 1.0 mM SNAP at 30 min, as shown by the arrow. The results are mean values ± SEM of 4–8 independent experiments

potentiation by DHPG was reduced by the antagonists RS-1-aminoindan-1,5-dicarbohydroxylate (AIDA, 0.1 mM) (Fig. 1A, Table 1) and L(+)-2-amino-3-phosphonopropionate (L-AP3, 0.1 mM) (Table 1).

The NOergic compounds affected the evoked release mainly in the adult hippocampus (Table 2). The NO-generating compounds sodium nitroprusside (SNP), S-nitroso-N-acetylpenicillamine (SNAP) and hydroxylamine (HA) reduced the release, as did also the NO synthase inhibitors 7-nitroindazole (7-NINA) and N^G-nitro-L-arginine (L-NNA) (Table 2). Figure 2 shows the effect of SNAP in the adult hippocampus as an example, since the time-courses of all the other NOergic compounds were very similar. The inhibition by SNP and SNAP was not affected by 7-NINA (Table 2).

The protein kinase C activator 4β-phorbol 12-myristate 13-acetate (PMA) potentiated the evoked release only in 7-day-old mice, this effect being reduced by the kinase antagonist 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7) (Fig. 1B). The adenosine compounds had effects only in the developing hippocampus (Fig. 3). The adenosine receptor A_1 agonists,

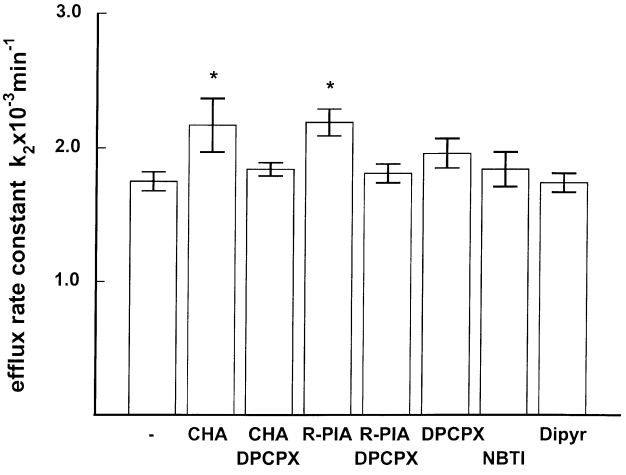

Fig. 3. Effect of adenosine compounds on the 0.1 mM NMDA-evoked taurine release from hippocampal slices from 7-day-old mice. The graph shows the efflux rate constants ± SEM ($k_2 \times 10^{-3}$ min^{-1}) for the time interval of 34–50 min. Number of independent experiments 4–8. Significance of differences from the control: *$p < 0.05$. *CHA*, N^6-cyclohexyladenosine; *DPCPX*, 8-cyclopentyl-1,3-dipropylxanthine; *R-PIA*, R(−)N^6-(2-phenylisopropyl)adenosine; *NBTI*, S-(4-nitrobenzyl)-6-thioinosine; *Dipyr*, dipyridamole

Table 1. Effects of glutamatergic compounds on the NMDA-evoked release of taurine from mouse hippocampal slices

Compound (mM)		Efflux rate constants k_2 (34–50 min) $\times 10^{-3}$ min^{-1} ± SEM	
		3-Month-old	7-Day-old
NMDA	0.1 (control)	1.78 ± 0.09 (7)	1.75 ± 0.07 (8)
Glutamate	0.1	1.76 ± 0.05 (4)	1.56 ± 0.05 (4)
Glutamate	1.0	2.01 ± 0.04 (4)	1.99 ± 0.09 (4)
AMPA	0.1	1.74 ± 0.18 (4)	2.16 ± 0.08** (8)
+NBQX	0.1	–	1.87 ± 0.05$^{\#}$ (4)
Kainate	0.1	1.58 ± 0.07 (4)	1.84 ± 0.13 (4)
Quisqualate	0.1	2.10 ± 0.28 (4)	1.80 ± 0.16 (4)
trans-ACPD	0.1	1.87 ± 0.11 (8)	2.01 ± 0.08* (4)
DHPG	0.1	1.72 ± 0.10 (8)	2.17 ± 0.08** (7)
+AIDA	0.1	–	1.79 ± 0.07$^{\#}$ (4)
+L-AP3	0.1	–	1.86 ± 0.05$^{\#}$ (4)
DCG IV	0.1	1.88 ± 0.15 (4)	1.83 ± 0.04 (4)
L-AP4	0.1	1.64 ± 0.06 (4)	1.94 ± 0.08 (4)
L-SOP	0.1	1.81 ± 0.14 (4)	1.62 ± 0.13 (4)
Dizocilpine	0.1	1.53 ± 0.05* (8)	1.30 ± 0.22* (4)

The slices were preloaded for 30 min with 10 μM [^{3}H]taurine in Krebs-Ringer-Hepes-glucose medium, pH 7.4, under O_2 and then superfused for 50 min with 0.1 mM NMDA (control). The other glutamatergic compounds were added at 30 min. The results are rate constants ± SEM for the period of 34–50 min (k_2). The rate constants k_2 for the efflux without NMDA were 1.54 ± 0.04 (n = 8) and 0.17 ± 0.02 (n = 13) $\times 10^{-3}$ min^{-1} in adults and 7-day-olds, respectively. Number of independent experiments in parenthesis. Significance of differences from the corresponding controls: * $p < 0.05$, ** $p < 0.01$, and from the release in the presence of DHPG or AMPA: $^{\#}$ $p < 0.05$. *AMPA*, 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; *trans-ACPD*, (1±)-1-aminocyclopentane-trans-1,3-dicarboxylate; *AIDA*, (RS)-1-aminoindan-1,5-dicarboxylate; *L-AP3*, L(+)-2-amino-3-phosphonopropionate; *DHPG*, (S)-3,5-dihydroxyphenylglycine; *DCG IV*, (2S, 2′R, 3′R)-2-(2′, 3′-dicarboxycyclopropyl)glycine; *L-AP4*, L(+)-2-amino-4-phosphonobutyrate; *NBQX*, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; *L-SOP*, O-phospho-L-serine

Table 2. Effects of NO compounds on the NMDA-evoked release of taurine from mouse hippocampal slices

Compound (mM)		Efflux rate constants k_2 (34–50 min) $\times 10^{-3}$ min^{-1} ± SEM	
		3-Month-old	7-Day-old
NMDA	0.1 (control)	1.78 ± 0.09 (7)	1.75 ± 0.07 (8)
SNP	1.0	1.23 ± 0.22* (4)	1.91 ± 0.05 (4)
+7-NINA	0.1	1.29 ± 0.07** (4)	–
SNAP	1.0	1.26 ± 0.11** (8)	1.52 ± 0.08* (6)
+7-NINA	0.1	1.39 ± 0.13* (4)	1.65 ± 0.03 (4)
HA	5.0	1.21 ± 0.14** (6)	1.90 ± 0.09 (8)
7-NINA	0.1	1.23 ± 0.13** (4)	1.79 ± 0.11 (4)
L-NNA	0.1	1.41 ± 0.09* (4)	1.78 ± 0.05 (4)
L-NNA	1.0	1.18 ± 0.05** (4)	1.69 ± 0.05 (4)

The experiments were carried out as in Fig. 1. The NO compounds were added at 30 min. Number of independent experiments in parenthesis. Significance of differences from the corresponding controls: * $p < 0.05$, ** $p < 0.01$. *SNP*, sodium nitroprusside; *SNAP*, S-nitroso-N-acetylpenicillamine; *HA*, hydroxylamine; *7-NINA*, monosodium salt of 7-nitroindazole; *L-NNA*, N^G-nitro-L-arginine

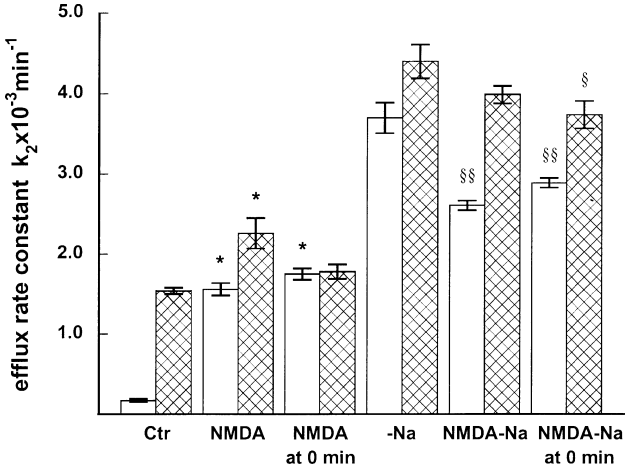

Fig. 4. Effects of Na^+-free media on the 0.1 mM NMDA-evoked taurine release from hippocampal slices from 7-day-old (open columns) and 3-month-old (cross-hatched columns) mice. The graph shows the efflux rate constants ± SEM ($k_2 \times 10^{-3}$ min^{-1}) for the time interval of 34–50 min. Number of independent experiments 4–8. *Ctr*, normal conditions (control); *NMDA*, 0.1 mM NMDA added at 30 min; *NMDA at 0 min*, 0.1 mM NMDA added at the beginning of superfusion; *−Na*, Na^+-free medium; *NMDA-Na*, 0.1 mM NMDA added at 30 min in Na^+-free medium; *NMDA-Na at 0 min*, Na^+-free medium and 0.1 mM NMDA added at the beginning of superfusion. The release was significantly ($p < 0.01$) increased in all cases by the omission of Na^+. Significance of the NMDA effects in Na^+-containing media: $^*p < 0.01$, and in Na^+-free media: $^§p < 0.05$, $^{§§}p < 0.01$

N^6-cyclohexyladenosine (CHA) and R(−)N^6-(2-phenylisopropyl)adenosine (R-PIA), significantly enhanced the NMDA-evoked release, the effect of R-PIA being antagonized by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (Fig. 3). The adenosine transport inhibitors S-(4-nitrobenzyl)-6-thioinosine (NTBI) and dipyridamole had no effects (Fig. 3).

Na^+-free medium greatly enhanced ($p < 0.01$) the basal taurine release in both age groups, when 0.1 mM NMDA at 30 min had no effect in adults but significantly ($p < 0.01$) reduced the release in developing mice (Fig. 4). When 0.1 mM NMDA was added to Na^+-free medium at the beginning of superfusion the release was reduced at both ages (Fig. 4).

Discussion

The NMDA-evoked release of taurine both in vivo and in vitro has been well documented, although very little is known about its regulation. For instance in vitro, NMDA has evoked taurine release from rat and mouse hippocampal slices (Magnusson et al., 1991; Menéndez et al., 1993; Saransaari and Oja, 1994, 1997b), mouse cerebral cortical slices (Saransaari and Oja, 1991) and cultured rat cerebellar granule cells (McCaslin and Yu, 1992). The evoked release is concentration-dependent, exhibiting an apparent maximum at a 0.1-mM concentration of NMDA in hippocampal slices from both developing and young adult mice (Saransaari and Oja, 1997b). In concert with the actions of NMDA, (RS)-(tetrazol-5-yl)glycine, a highly potent NMDA receptor agonist, has likewise enhanced taurine release from mouse hippocampal slices (Saransaari and Oja, 1994). NMDA is a markedly more powerful effector in the developing than in the adult mouse hippocampus (Saransaari and Oja, 1997b). Similarly, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG IV), which is an agonist of the group II metabotropic glutamate receptors but at high concentrations activates the NMDA receptors (Uyama et al., 1997), has markedly enhanced taurine release only from hippocampal slices from developing mice (Saransaari and Oja, 1999a). The efficacy of NMDA to enhance taurine release from hippocampal slices declines rapidly with age in developing mice, tallying with the transient overexpression of the NMDA class of glutamate receptors during postnatal development (McDonald et al., 1990; Le Grevés et al., 1996). The increase in the number of receptor sites also correlates with the development of afferent input and elaboration of dendrites in the hippocampus and with the developmental onset of long-term potentiation (Pokorny and Yamamoto, 1981).

The properties of the NMDA-evoked release of taurine were first characterized using specific NMDA antagonists. The NMDA- and tetrazolylglycine-evoked releases from hippocampal slices (Saransaari and Oja, 1994, 1997b; this study) are blocked by dizocilpine, the potent non-competitive NMDA receptor antagonist. The enhancing effects are thus receptor-mediated (Saransaari and Oja, 1997b). Presynaptic glutamate receptors also regulate the release of transmitters in the hippocampus in vitro, for example, that of glutamate (Smirnova et al., 1993), noradrenaline (Pittaluga and Raiteri, 1992) and GABA (Janáky et al., 1993).

Electrophysiological and immunochemical data suggest that alterations in the function of one glutamate receptor subtype may affect the function of other subtypes (He et al., 1998) and that the receptors may interact with each other (Healy and Meador-Woodruff, 2000). Our results indicate that the NMDA-

evoked taurine release is also enhanced by AMPA receptor activation in the immature hippocampus. Moreover, metabotropic group I glutamate receptors appear to regulate the NMDA-evoked release in the hippocampus of developing mice, since the agonists DHPG and trans-ACPD potentiated the release in a receptor-mediated manner. In vitro studies with brain slice and synaptosomal preparations have demonstrated the existence of presynaptic metabotropic glutamate receptors (Herrero et al., 1994; Lombardi et al., 1996). Group I receptors are known to increase neuronal excitation and excitability (see Nicoletti et al., 1996). Indeed, the receptors of group I have been shown to synergize with NMDA receptors in inducing neuronal damage (Sacaan and Schoepp, 1992), and quisqualate and DHPG to enhance NMDA toxicity in cultured neurons (Buisson and Choi, 1995). In the hippocampus, group I metabotropic receptors also inhibit synaptic transmission through a presynaptic mechanism (Manzoni and Bockaert, 1995). The taurine release enhanced by NMDA receptor and metabotropic group I receptor activation may reduce hyperexcitation or strengthen the inhibitory effects, being thus neuroprotective in either case.

NO modulates the NMDA-evoked release of neurotransmitters in the brain, e.g., that of GABA and monoamines (Segeith et al., 1995; Silva et al., 1995; Getting et al., 1996; Kendrick et al., 1996; Kaehler et al., 1999; Smith and Whitton 2000). The production of NO is linked to the activation of NMDA receptors (Schuman and Madison, 1994), the presynaptically released glutamate activating postsynaptic NMDA receptors. The receptor-linked ion channel allows Ca^{2+} to enter the cell upon depolarization, which activates a Ca^{2+}-dependent enzyme, NO synthase (Bredt et al., 1992). The NO formed may act intracellularly or diffuse out and act extracellularly at the soluble guanylate cyclase to enhance the content of 3′,5′-cyclic guanosine monophosphate (cGMP) (Knowles et al., 1989). We have recently demonstrated that NO-generating compounds modulate taurine release in both immature and mature hippocampus, suggesting the involvement of NO-mediated processes in taurine release under both normal (Saransaari and Oja, 1999b) and ischemic conditions (Saransaari and Oja, 2002b). Furthermore, the basal taurine release is affected by the cGMP level in both adult and developing hippocampus (Saransaari and Oja, 2002a). The NMDA-evoked taurine release was now inhibited by the NO synthase inhibitors nitroarginine and 7-nitroindazole only in the adult hippocampus. The NO/cGMP pathway is thus involved in this release. The NMDA-induced taurine release has also been inhibited by the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) in the rat cerebral cortex in vivo (Scheller et al., 2000). NO also mediates NMDA-evoked GABA release from chick retina cells (Ientile et al., 1997).

The inhibitory effects of the NO generators SNAP, SNP and HA are controversial, indicating complex interactions of NO with the release. It should be borne in mind, however, that there are different cell types and transmitter systems in hippocampal slices. GABA strongly controls glutametergic circuits in the hippocampus and the basal levels of NO and cGMP appear to be under tonic inhibitory GABAergic regulation (Fedele et al., 2001). One mM SNAP has been shown to enhance GABA release in the hippocampus (Getting et al., 1996) and striatum (Trabace and Kendrick, 2000), and particularly $GABA_B$ receptor activation inhibits the stimulated release of taurine in the adult hippocampus (Saransaari and Oja, 2000a). The direct stimulatory effect of NO donors on the NMDA-stimulated taurine release, not affected by NO synthase inhibitors, is then nevertheless overshadowed by indirect inhibition through GABAergic connections. The location and timing of NO synthesis are also apparently important for the effects of NO to manifest themselves (Izumi et al., 1992). Although NO is a short-lived messenger in nervous tissue, the onset of responses seems to be fairly slow in taurine release (Saransaari and Oja, 1999b).

Activation of presynaptic protein kinase C enhances the release of neurotransmitters from nerve terminals (Eboli et al., 1993; Ohtani et al., 1995) and potentiates synaptic transmission in the hippocampus (Malenka et al., 1986). The protein kinase C family is involved in the regulation of synaptic plasticity and gene expression. Furthermore, it has also been implicated in the cellular processes leading to neurodegeneration arising from cerebral ischemia (Durkin et al., 1996). Protein kinase C activation by phorbol esters has been shown to enhance NMDA currents in various preparations (Cheng and Huang, 1991; Kelso et al., 1992) and to potentiate the NMDA-evoked release of adenosine and norepinephrine from rat cortical slices (Wang and White, 1998). Activation of protein kinase C by PMA did not now affect the NMDA-stimulated taurine release in the adult hippocampus. In the immature mice, the PMA-stimulated release was reduced by the

kinase inhibitor H-7, thus reflecting the involvement of protein kinase C in the release. The basal taurine release from hippocampal slices from adult mice has also been affected by protein kinase C activation (Saransaari and Oja, 2002b), whereas the release from glial cells in the rat supraopticus nucleus has not been affected by inhibitors and activators of protein kinases (Deleuse et al., 2000). 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 neuromodulator adenosine is known to inhibit the presynaptic release of neurotransmitters, including glutamate (Fredholm and Dunwiddie, 1988; Heron et al., 1993), and to hyperpolarize postsynaptic neurons in the hippocampus (Greene and Haas, 1991). These findings corroborate the view that adenosine is an endogenous protective agent against cerebral ischemia and excitotoxic neuronal damage (Deckert and Gleiter, 1994). Four types of adenosine receptors have been identified, A_1, A_{2A}, A_{2B} and A_3, the A_1 receptors being particularly involved in the regulation of neurotransmitter release (Fredholm et al., 1994). We have recently shown that the basal release of taurine is modulated by A_1 receptors in both mature and immature hippocampus, whereas in ischemia these receptors potentiate taurine release only in adults (Saransaari and Oja, 2000c). On the other hand, the NMDA-stimulated taurine release was now modulated by adenosine receptors only in the developing hippocampus, the enhancing effect being again mediated by the A_1 receptors. The elevated taurine levels together with the depression of excitatory amino acid release by adenosine receptor activation could be beneficial under ischemic conditions, protecting neural cells against excitotoxicity and hyperexcitation.

The release of taurine induced by NMDA receptor activation is largely dependent on Ca^{2+} mobilization from intracellular stores, since removal of extracellular taurine diminishes, but does not abolish, the extracellular increase in taurine in the rat hippocampus (Menéndez et al., 1993). The influx of extracellular Ca^{2+} may thus provide the signal for the NMDA-evoked release of taurine, which is amplified by Ca^{2+}-dependent Ca^{2+} mobilization from intracellular stores. The NMDA-evoked release from hippocampal slices is largely dependent on extracellular Ca^{2+} in adult mice, whereas this release and its antagonism by dizocilpine are marked without extracellular Ca^{2+} in developing mice (Saransaari and Oja, 1997b). Both effects were also accentuated in developing mice in the absence of extracellular Mg^{2+}.

Taurine release was now markedly increased in the absence of Na^+ from the superfusion medium. This may result from changes in the operation of Na^+-dependent, carrier-mediated cell membrane transport (Saransaari and Oja, 1992). When the normal Na^+ gradient is abolished, the preferred direction of transport changes from inward (uptake) to outward (release). In Na^+-free media brain slices also lose intracellular K^+ (Korpi and Oja, 1983). Under these circumstances any presynaptic NMDA effects could have been overridden by the massive release of taurine via plasma membrane carriers. So was the case in adult mice, but in developing mice other factors must also intervene, as the release in Na^+-free media was even attenuated by NMDA. This effect also shows that Na^+-free media may not only increase simple physical diffusion of taurine through cell membranes.

As a conclusion, the release of taurine stimulated by NMDA is receptor-mediated in both adult and developing mouse hippocampus. In adults, only NOergic compounds affected the evoked release, indicating that the release is mediated by the NO/cGMP pathway. On the other hand, the regulation of the NMDA-evoked taurine release seems to be somewhat complex in the immature hippocampus. The release was shown to be further potentiated by activation of protein kinase C and adenosine A_1 receptors as well as ionotropic AMPA receptors and metabotropic glutamate group I receptors. The NMDA-receptor-stimulated taurine release could be neuroprotective, being particularly important under cell-damaging conditions in the developing hippocampus.

Acknowledgments

The skillful technical assistance of Mrs Irma Rantamaa, Mrs Oili Pääkkönen, Mrs Sari Luokkala and the financial support of the Medical Research Fund of Tampere University Hospital are gratefully acknowledged.

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