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Involvement of metabotropic glutamate receptors in taurine release in the adult and developing mouse hippocampus

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Summary. The inhibitory amino acid taurine has been held to function as an osmoregulator and modulator of neural activity, being particularly important in the immature brain. Ionotropic glutamate receptor agonists are known markedly to potentiate taurine release. The effects of different metabotropic glutamate receptor (mGluR) agonists and antagonists on the basal and K^+-stimulated release of [^{3}H]taurine from hippocampal slices from 3-month-old (adult) and 7-day-old mice were now investigated using a superfusion system. Of group I metabotropic glutamate receptor agonists, quisqualate potentiated basal taurine release in both age groups, more markedly in the immature hippocampus. This action was not antagonized by the specific antagonists of group I but by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX), which would suggest an involvement of ionotropic glutamate receptors. (S)-3,5-dihydroxyphenylglycine (DHPG) potentiated the basal release by a receptor-mediated mechanism in the immature hippocampus. The group II agonist (2S, 2′R, 3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG IV) markedly potentiated basal taurine release at both ages. These effects were antagonized by dizocilpine, indicating again the participation of ionotropic receptors. Group III agonists slightly potentiated basal taurine release, as did several antagonists of the three metabotropic receptor groups. Potassium-stimulated (50 mM K^+) taurine release was generally significantly reduced by mGluR agents, mainly by group I and II compounds. This may be harmful to neurons in hyperexcitatory states. On the other hand, the potentiation by mGluRs of basal taurine release, particularly in the immature hippocampus, together with the earlier demonstrated pronounced enhancement by activation of ionotropic glutamate receptors, may protect neurons against excitotoxicity.

Keywords: Amino acids – Taurine release – Metabotropic glutamate receptors – Hippocampal slices – Adult – Developing mice

Abbreviations: ACPD: (1±)-1-aminocyclopentane-trans-1,3-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; DCG IV: (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine; DHPG: (S)-3,5-dihydroxyphenylglycine; EGLU: (2S)-2-ethylglutamate; L-AP3: L(+)-2-amino-3-phosphonopropionate; L-AP4: L(+)-2-amino-4-phosphonobutyrate; L-AP6: L(+)-2-amino-6-phosphonohexanoate; L-SOP: O-phospho-L-serine; MPPG: (RS)-2-methyl-4-phosphonophenylglycine; MSOP: (RS)-2-methylserine-O-phosphate; MSOPPE: (RS)-2-methylserine-O-phosphate monophenyl ester; MTPG: (RS)-2-methyl-4-tetrazolylphenylglycine; NBQX: 6-nitro-7-sulphamoyl[f]quinoxaline-2,3-dione; NMDA: N-methyl-D-aspartate; QA: quisqualate; S-3C4H-PG: (S)-3-carboxy-4-hydroxyphenylglycine; S-4C-PG: (S)-4-carboxyphenylglycine; S-MCGP: (S)-2-methyl-4-carboxyphenylglycine.

Introduction

The inhibitory amino acid taurine has been thought to function as a regulator of neuronal activity, particularly in the immature brain (Kontro and Oja, 1987; Huxtable, 1992; Sturman, 1993). The involvement of taurine in osmoregulation and cell volume adjustments in the central nervous system has also been well documented (Solis et al., 1988; Pasantes-Morales and Schousboe, 1989; Oja and Saransaari, 1996). Moreover, taurine protects neural cells from excitotoxicity induced by excitatory amino acids in the hippocampus (French et al., 1986), cerebellum (Trenkner, 1990) and neuronal cell cultures (Tang et al., 1996), forestalls harmful metabolic events evoked by ischemia or hypoxia (Schurr et al., 1987) and ameliorates symptoms in epilepsy (Oja and Kontro, 1983). The hippocampus is involved in many important brain functions including generation of long-term potentiation, memory formation, learning, arousal, emotions and regulation of autonomic functions. The major part of excitatory innervation in the hippocampus, including pyramidal cells, is glutamatergic. The function of these neurons is modulated by inhibitory GABA-releasing interneurons (Frotscher et al., 1984; Freund and Buzsági, 1988). The structural analogue of GABA, taurine, also abounds in the hippocampus (Kontro et al., 1980; Palkovits et al., 1986) and taurine-like immunoreactivity has been located in hippocampal interneurons, pyramidal neurons and dentate granule cells (Magnusson et al., 1989). Taurine inhibits the firing of hippocampal pyramidal neurons by increasing membrane chloride conductance and causing hyperpolarization (Taber et al., 1986). The taurine-synthesizing enzyme, cysteine sulphinate decarboxylase, has also been identified in pyramidal basket interneurons (Taber et al., 1986).

It has recently been demonstrated that the ionotropic glutamate receptor agonists N-methyl-D-aspartate (NMDA), kainate and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) evoke taurine release in both the adult and the developing hippocampus (Magnusson et al., 1991; Saransaari

and Oja, 1996; 1997a,b), the effects being more pronounced in the latter (Saransaari and Oja, 1997a). The hippocampal innervation also includes metabotropic glutamate receptors (mGluRs), a large family of receptors coupled to second-messenger systems via GTP-binding proteins. At least eight mGluR subtypes have been cloned to date, and these receptors can be divided into three major groups based on their pharmacology, second-messenger coupling and sequence homology (see Pin and Duvoisin, 1995; Conn and Pin, 1997). Receptor subtypes $mGluR_1$ and $mGluR_5$, which make up group I, are coupled to a G-protein which activates phospholipase C, initiating phosphoinositole hydrolysis. Receptors $mGluR_2$ and $mGluR_3$ (group II) are negatively coupled to the adenylate cyclase system, as are also receptors $mGluR_4$, $mGluR_6$, $mGluR_7$, and $mGluR_8$, making up group III. The metabotropic glutamate receptors are involved in a variety of physiological functions in the central nervous system, particularly in the processes of neuromodulation and synaptic plasticity (see Riedel, 1996; Conn and Pin, 1997). So far no data are available as to the possible involvement of metabotropic glutamate receptors with the functions of taurine. These interactions could be of great importance, however, in view of the protective effects of taurine in the hippocampus. We now studied the actions of various agonists and antagonists of metabotropic glutamate receptors on the release of preloaded [^{3}H]taurine from hippocampal slices from 3-month-old (adult) and 7-day-old mice, using a superfusion system.

Materials and methods

Material

NMRI mice of both sexes aged 3 months (adults) and 7 days were used throughout. [1,2-^{3}H]Taurine (specific radioactivity 1.07 PBq/mol) was obtained from Amersham International, Bristol, UK. All other drugs were from Tocris Cookson, Bristol, UK.

Efflux experiments

Slices 0.4 mm thick weighing 15–20 mg were prepared from the hippocampi with a Stadie-Riggs tissue slicer and used immediately in efflux experiments. The slices were first preloaded for 30 min with 10 μM (50 MBq/l) [^{3}H]taurine in preoxygenated Krebs-Ringer-Hepes-glucose medium (pH 7.4) under O_2 and superfused as described in detail in Kontro and Oja (1987). 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 in many experiments changed to another 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 likewise counted for radioactivity.

Estimation of efflux rate constants

The desaturation curves of labelled 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 to 30 min (k_1) and 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

The basal release of [^{3}H]taurine from hippocampal slices from adult mice was not affected by the group I metabotropic glutamate receptor agonists (1±)-1-aminocyclopentane-trans-1,3-dicarboxylate) (trans-ACPD) and (S)-3,5-dihydroxyphenylglycine (DHPG) (both 0.1 mM) (data not shown). Quisqualate (0.1 mM) stimulated the release; more effectively in the immature than in the adult hippocampus (Fig. 1). The effect was clearly concentration-dependent at a 0.1 μM–500 μM concentration of quisqualate (Fig. 1). The action of quisqualate (0.1 mM) was not affected by the group I antagonists, (S)-2-methyl-4-carboxyphenylglycine (S-MCGP), (RS)-1-aminoindan-1,5-dicarboxylate (AIDA), (S)-4-carboxyphenylglycine [(S)-4C-PG] and L(+)-2-amino-3-phosphonopropionate (L-AP3) (all 0.1 mM) and by the ionotropic receptor antagonists (6-cyano-7-nitroquinoxaline-2,3-dione) (CNQX) and 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX) in the adult hippocampus (data not shown). In the immature hippocampus CNQX and NBQX reduced the quisqualate-stimulated release, but did not abolish it (Fig. 2A). Trans-ACPD (0.1 mM) had no effect in the immature hippocampus

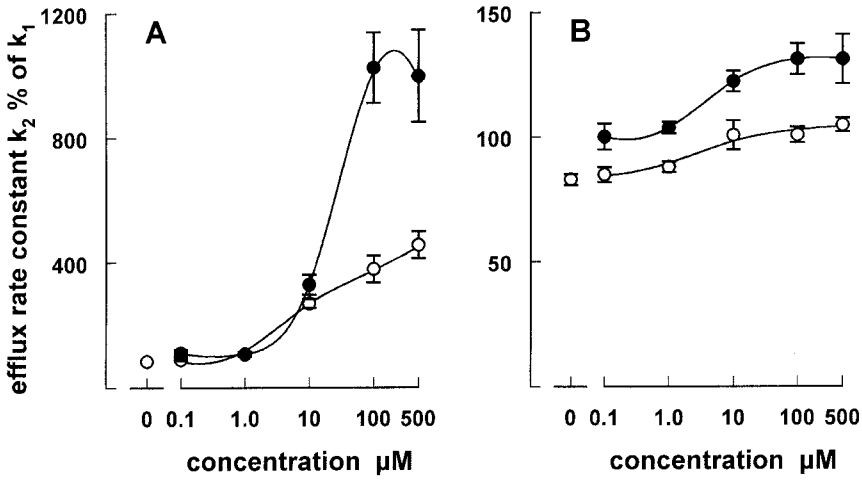

Fig. 1. Concentration-dependence of the stimulation of taurine release by quisqualate (–○–) and DCG IV (–●–) from hippocampal slices from 7-day-old (**A**) and 3-month-old (**B**) mice. The results are efflux rate constants k_2 (34–50 min) ± SEM as percentages of the corresponding rate constants k_1 (20–30 min). Number of independent experiments 4–8

(data not shown), but DHPG (0.1 mM) potentiated the basal release by about 28% (Fig. 2B). The effect of DHPG was significantly reduced by 0.1 mM AIDA and S-MCGP (Fig. 2B). Moreover, the antagonists AIDA and (S)-4C-PG (both 0.1 mM) slightly stimulated taurine release in the adults, whereas L-AP3, AIDA and (S)-3-carboxy-4-hydroxyphenylglycine [(S)-3C4H-PG] were effective in the 7-day-olds (Fig. 3).

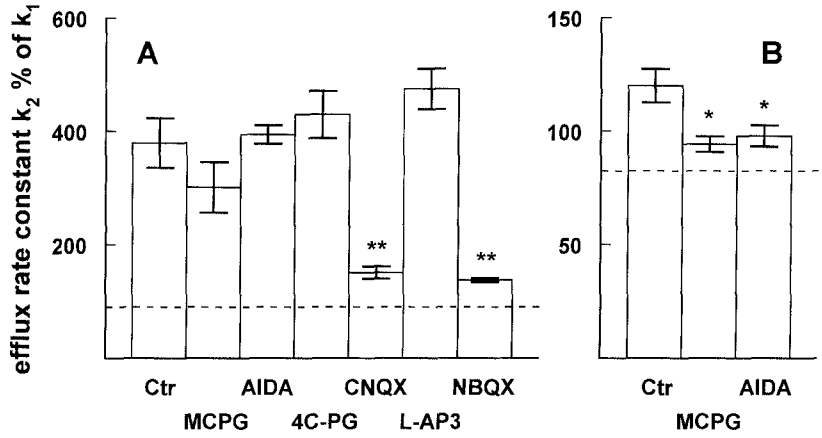

Fig. 2. Effects of the antagonists of glutamate receptors (all 0.1 mM) on the 0.1 mM quisqualate-(*Ctr*) (**A**) and 0.1 mM DHPG-(*Ctr*) (**B**) stimulated taurine release from the hippocampi of 7-day-old mice. The bars depict the efflux rate constants k_2 (34–50 min) ± SEM as percentages of the rate constants k_1 (20–30 min). Number of independent experiments 4–8. Significance of differences from the control: *$p < 0.01$. For the names of the substances see Abbreviations. The dashed lines indicate the basal unstimulated release

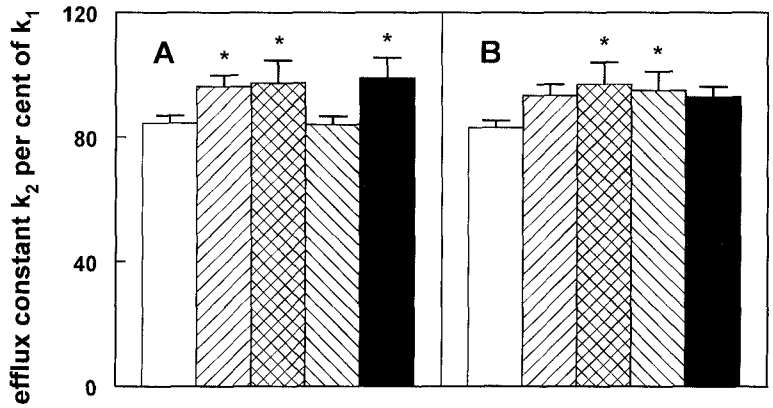

Fig. 3. Effects of antagonists (all 0.1 mM) of group I metabotropic glutamate receptors on hippocampal taurine release in 7-day-old (**A**) and 3-month-old (**B**) mice. The bars depict the efflux rate constants k_2 (34–50 min) ± SEM as percentages of the rate constants k_1 (20–30 min). The bars are □ control, ▨ L-AP3, ▩ AIDA, ▧ S-4C-PG, ■ S-3CH-PG (see Abbreviations). Number of independent experiments 4–8. Significance of differences from the corresponding control: *$p < 0.05$

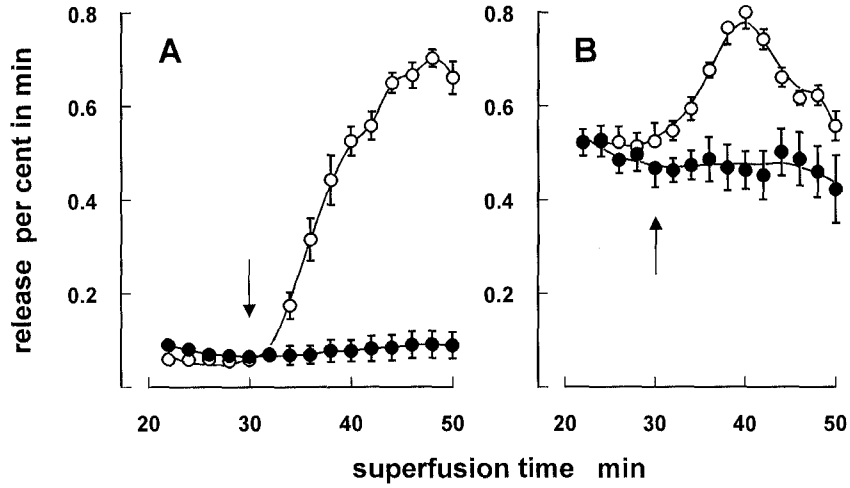

Fig. 4. Taurine release from hippocampal slices from 7-day-old (**A**) and 3-month-old (**B**) mice in the presence of 0.1 mM DCG IV (–○–) and DCG IV together with dizocilpine (0.1 mM) (–●–). The drugs were added to the superfusion medium at 30 min as indicated by the arrow. The results are mean values ± SEM of 4–8 separate experiments

Of the group II metabotropic glutamate receptor agonists, (S)-4C-PG (0.1 mM) had no effect on the basal taurine release, but (2S,2'R,3'R)-2-(2', 3'-dicarboxycyclopropyl)glycine (DCG IV) markedly and concentration-dependently potentiated the release in both adult and developing hippocampus (Fig. 1). This effect was striking in the 7-day-olds. The potentiation by 0.1 mM DCG IV was almost totally abolished by 0.1 mM dizocilpine in both age groups studied (Fig. 4), while the antagonists (RS)-2-methyl-4-tetrazolylphenylglycine (MTPG), (2S)-2-ethylglutamate (EGLU), S-MCPG and (RS)-2-methylserine-O-phosphate monophenyl ester (MSOPPE) (all 0.1 mM) had no effect on the DCG IV -stimulated release (data not shown). Of these antagonists, 0.1 mM EGLU stimulated basal taurine release by 18.4 ± 0.6% and 32.8 ± 0.5% (mean ± SEM, n = 4) in the adult and developing hippocampus, respectively. Furthermore, 0.1 mM MSOPPE potentiated the basal release by 38.1 ± 2.9% (mean ± SEM, n = 4) in the immature hippocampus and 0.1 mM S-MCPG by 26.0 ± 1.6% (mean ± SEM, n = 4) in the adult animals.

The group III agonist L(+)-2-amino-4-phosphonobutyrate (L-AP4) (0.1 mM) slightly stimulated (14.4 ± 0.2%, mean ± SEM, n = 4) the basal taurine release in the immature hippocampus. Another group III agonist O-phospho-L-serine (L-SOP) (0.1 mM) also significantly potentiated the release at both ages (Fig. 5), which effects were not significantly modified by the antagonists (RS)-2-methyl-4-phosphonophenylglycine (MPPG) and (RS)-2-methylserine-O-phosphate (MSOP) (both 0.1 mM). The antagonist (RS)-2-cyclopropyl-4-phosphonophenylglycine (CPPG) (0.1 mM) abolished the action of L-SOP in the adults, but not in the developing mice (Fig. 5). Of these antagonists, 0.1 mM MPPG and MSOP, when applied alone, stimulated ($p <$

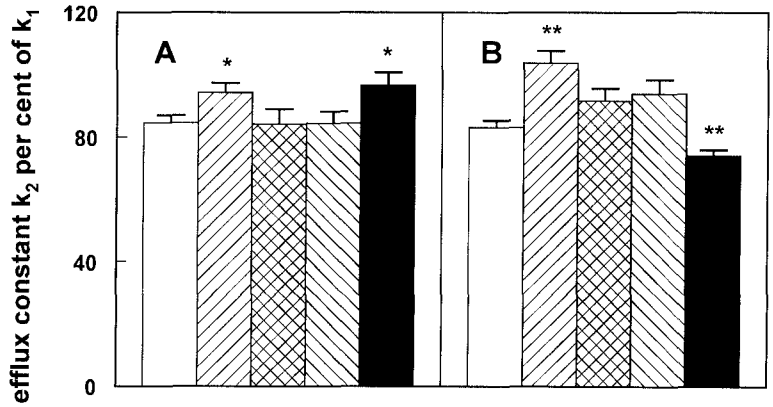

Fig. 5. Effects of antagonists (all 0.1 mM) of group III metabotropic glutamate receptors on hippocampal taurine release enhanced by L-SOP (0.1 mM) in 7-day-old (**A**) and 3-month-old (**B**) mice. The bars depict the efflux rate constants k_2 (34–50 min) ± SEM as percentages of the rate constants k_1 (20–30 min). The bars are ☐ basal release, ▨ L-SOP alone (control), ▩ L-SOP + MPPG, ▧ L-SOP + MSOP, ■ L-SOP + CPPG (see Abbreviations). Number of independent experiments 4–8. Significance of differences from the corresponding controls: *$p < 0.05$, **$p < 0.01$

0.01) basal taurine release in the adults, 19.3 ± 0.3% (mean ± SEM, n = 8) and 17.8 ± 0.3% (mean ± SEM, n = 4), respectively.

Potassium stimulation by 50 mM K^+ potentiated hippocampal taurine release about 2-fold in the adults and 8-fold in the 7-day-olds (Table 1). The group I metabotropic agonists trans-ACPD and quisqualate did not affect this stimulated release, but the antagonists S-3CH-PG, S-4C-PG (both 0.1 mM) and AIDA (0.1 and 0.01 mM) significantly reduced it in the adult hippocampus and S-3CH-PG and S-4C-PG (both 0.1 mM) in the immature animals (Table 1). A lower concentration (0.01 mM) of 3CH-PG and S-4C-PG had no effect on the stimulated release. DCG IV (0.1 mM) almost doubled the K^+-stimulated release in the developing hippocampus without any effect in the adults (Table 1). Furthermore, of the drugs acting at the group III receptors, MSOP significantly potentiated stimulated taurine release in the 7-day-olds, while L-SOP, MPPG and MSOP (all 0.1 mM) inhibited this release in the adults (Table 1).

Discussion

In situ localization of mRNAs encoding the different mGluRs has shown that all subtypes are present in the hippocampus (Abe et al., 1992; Shigemoto et al., 1992; 1996; Fotuhi et al., 1994; Nakanishi et al., 1994), demonstrating differential presynaptic localization. Presynaptic mGluRs regulating the release of excitatory amino acids have been found in both *in vitro* and *in vivo* release studies in various brain-slice and synaptosomal preparations (Herrero et al., 1994; Lombardi et al., 1994; 1996; East et al., 1995). The compounds affecting mGluRs were also now able to modify hippocampal taurine release

Table 1. Effects of agonists and antagonists of metabotropic glutamate receptors on the potassium-stimulated taurine release in mouse hippocampal slices

Substance (mM)	Efflux rate constants k_2 (34–50 min) (% of k_1)	
	7-day-old	3-month-old
None (control)	638.9 ± 62.1 (16)	159.0 ± 6.2 (10)
Group I		
trans-ACPD 0.1	460.9 ± 41.9 (8)	150.2 ± 11.7 (4)
QA 0.1	614.6 ± 45.5 (4)	141.9 ± 4.1 (4)
S-3CH-PG 0.1	369.4 ± 43.4* (4)	132.4 ± 7.7* (4)
S-3CH-PG 0.01	635.7 ± 31.0 (4)	–
S-4C-PG 0.1	294.2 ± 14.1* (4)	127.7 ± 4.7* (4)
S-4C-PG 0.01	641.8 ± 36.8 (4)	–
AIDA 0.1	664.6 ± 69.6 (4)	121.4 ± 4.1** (7)
AIDA 0.01	–	120.7 ± 5.0** (4)
DHPG 0.1	584.6 ± 58.1 (4)	162.1 ± 4.1 (4)
Group II		
DCG IV 0.1	1377.6 ± 88.3** (6)	150.6 ± 9.7 (4)
DCG IV 0.01	476.0 ± 51.1 (6)	149.4 ± 6.8 (4)
MSOPPE 0.1	776.5 ± 62.2 (4)	145.7 ± 6.8 (4)
Group III		
L-AP4 0.1	537.9 ± 56.6 (12)	137.0 ± 12.6 (8)
LSOP 0.1	826.9 ± 61.5 (4)	126.2 ± 5.0* (4)
LSOP 0.01	–	142.5 ± 14.3 (4)
MPPG 0.1	769.2 ± 86.3 (4)	124.3 ± 9.8* (5)
MPPG 0.01	–	122.9 ± 7.2* (4)
MSOP 0.1	932.2 ± 98.8* (4)	125.1 ± 8.9* (4)
MSOP 0.01	–	128.4 ± 1.9* (4)

The slices were preloaded for 30 min in Krebs-Ringer-Hepes-glucose medium, pH 7.4, with 10 μM [^{3}H]taurine and then superfused for 50 min, from 30 min onwards with 50 mM K^+ together with the above substances. The results are percentages of the basal efflux rate constant (k_1 20–30 min) of each slice. Number of independent experiments in parentheses. Significance of differences from the corresponding controls: *$p < 0.05$, **$p < 0.01$. For the names of the substances used see Abbreviations.

in both the adult and the developing hippocampus, although the actions were not so pronounced as those of ionotropic glutamatergic agents (Magnusson et al., 1991; Saransaari and Oja, 1996; 1997a). The activation of NMDA and AMPA classes of glutamate receptors is involved in hippocampal taurine release throughout the life-span of mice, while the kainate receptor-mediated release does not function in adults (Saransaari and Oja, 1997a). The variability now observed in the responses of taurine release to the metabotropic glutamatergic agents might be due both to the heterogeneity of the mGluRs and the considerable overlap of the selectivity of the drugs used (Schoepp and Conn, 1993; Pin and Duvoisin, 1995; Conn and Pin, 1997). Moreover, there may occur cross-talk between the different receptor subtypes, as has been shown for example between group I and II receptors in the neonatal rat cortex

(Schaffhauser et al., 1997), which complicates interpretations. In addition to this, a developmental change from inhibition to facilitation has been demonstrated in the control of glutamate release by mGluRs, nerve terminals from young animals exhibiting both inhibitory and facilitatory pathways (Herrero et al., 1996; 1998).

In general, the metabotropic glutamatergic agents may modify taurine release by means of at least three mechanisms. First, the activation of presynaptic heteroreceptors on nerve terminals could affect taurine release in the same manner as presynaptic mGluRs reduce GABA release and inhibitory synaptic transmission in several brain areas (Hayashi et al., 1993; Desai et al., 1994; Salt and Eaton, 1995; Schaffhauser et al., 1998), including the hippocampus (Gereau and Conn, 1995; Poncer et al., 1995). Second, the activation of glutamate autoreceptors enhances glutamate release, which could then subsequently evoke taurine release through the ionotropic receptors. Third, multisynaptic mechanisms may also be involved. In this case the agonists increase the firing rate of local circuit neurons or activate feedback loops, indirectly stimulating or inhibiting taurine release.

Of the group I agonists, quisqualate is also a potent AMPA receptor agonist (Schoepp et al., 1990) and may also mediate a persistent sensitization of L(+)-2-amino-6-phosphonohexanoate (L-AP6) and L-AP4 ('quis-effect') by its own receptor type coupled to phosphoinositol hydrolysis (Littman et al., 1995). This drug potentiated taurine release in the adult hippocampus, which action was not mediated by glutamate receptors, since the antagonists of both metabotropic and ionotropic receptors were not able to reduce it. The pronounced, concentration-dependent enhancement of taurine release by quisqualate in the immature hippocampus is not mediated by metabotropic receptors. The antagonism by CNQX and NBQX bespeaks the involvement of ionotropic receptors in the release. On the other hand, the stimulation of release by DHPG in the developing hippocampus was reduced by these antagonists, being thus apparently mediated by group I metabotropic receptors. DHPG has been described as a selective agonist of the metabotropic glutamate receptor coupled to phospholipase C (Thomsen et al., 1994), effectively stimulating phosphoinositide hydrolysis in different brain regions also in the developing brain (Schaffhauser et al., 1997; Sacaan et al., 1998). The level of $mGluR_1$ expression gradually increases in the brain during early postnatal development with the maturation of neuronal elements (Shigemoto et al., 1992). Moreover, the expression of $mGlu_{5a}$-receptor mRNA is higher in early postnatal life than in adults, where $mGlu_{5b}$-receptor mRNA is predominant (Minakami et al., 1995). The observed DHPG effect in the immature hippocampus could thus be due to this developmental overexpression of $mGluR_1$ receptors.

Group I receptors are known generally to increase neuronal excitation and excitability (see Nicoletti et al., 1996). Indeed, the mGluRs of group I have been shown to synergize with NMDA receptors in inducing neuronal damage (McDonald and Schoepp, 1992; Sacaan and Schoepp, 1992) and quisqualate and DHPG enhance NMDA toxicity in cultured neurons (Buisson and Choi, 1995). At variance with these findings group I mGluRs

also inhibit synaptic transmission in the hippocampus through a presynaptic mechanism (Gereau and Conn, 1995; Manzoni and Bockaert, 1995). Thus, the enhanced taurine release by mGluR I activation may reduce hyperexcitation or strengthen the inhibitory effects, being thus in both cases neuroprotective. Although these effects are not very marked they may nevertheless contribute to neuroprotection due to the considerable enhancement of taurine release evoked by the ionotropic receptors in cell-damaging conditions (Saransaari and Oja, 1997b), particularly in the immature hippocampus.

The activation of group II and III mGluRs generally reduces synaptic excitation and it has been suggested that they function as inhibitory autoreceptors (see Nicoletti et al., 1996; Sánchez-Prieto et al., 1996). For example, the mGluR II receptors tonically inhibit glutamate release from corticostriatal terminals (Cozzi et al., 1997). The agonists of both group II and III receptors are neuroprotective. DCG IV and other agonists protect cultured neurons against degeneration induced by excitotoxic concentrations of NMDA or kainate (Bruno et al., 1994; 1995). The activation of mGluRs of groups II and III by L-AP4 and L-SOP also produces neuroprotective effects in neurons and brain slices (Bruno et al., 1995; Maiese et al., 1995). The marked concentration-dependent stimulation of basal taurine release by DCG IV appears not to be mediated by the activation of group II receptors, since their specific antagonists failed to have any effect. In contrast, dizocilpine, the potent NMDA receptor antagonist, almost blocked the DCG IV effect in both age groups studied, indicating the involvement of ionotropic receptors. Indeed, DCG IV is known to behave as an NMDA receptor agonist, activating the NMDA-sensitive receptors at concentrations higher than 10μM (Wilsch et al., 1994; Uyama et al., 1997). The NMDA-evoked taurine release is strikingly large in the immature hippocampus (Saransaari and Oja, 1997a), which would corroborate the suggestion that ionotropic receptors participate in this release.

An inhibitory presynaptic mGluR sensitive to the group III agonist L-AP4 has been described in synaptosomal preparations (Jones and Roberts, 1990; Vazquez et al., 1995), consistent with the developmentally regulated depression of synaptic transmission by L-AP4 in the hippocampus (Baskys and Malenka, 1991). In accordance, the Ca^{2+}-dependent release of glutamate has been reduced by L-AP4 in a concentration-dependent manner (Herrero et al., 1996). The small stimulation of taurine release in the immature hippocampus by both L-AP4 and L-SOP may contribute to the above depression, though the effects seem not to be receptor-regulated. On the other hand, in the adults the L-SOP effect may be mediated by group III receptor activation, since the antagonist CPPG totally abolished it. Moreover, suppression of the activities of both group II and III receptors by their respective antagonists also slightly stimulated taurine release in the adults. Taken together, however, the small potentiations of taurine release by both group II and III receptors seem to be of minor importance in regulating hippocampal excitability. The small effects on the release could be due to inhibition of glutamate release by the autoreceptors.

While the basal taurine release was generally potentiated by different mGluRs, the potassium-stimulated release was inhibited by several mGluR agonists, group I and III compounds being active in this respect. A reduction in the evoked release of glutamate and aspartate by group II and III receptor activation has been observed in some brain preparations *in vitro* and *in vivo* (Lombardi et al., 1993; 1994; 1995; East et al., 1995; Battaglia et al., 1997; Lada et al., 1998). These findings are in keeping with a number of previous reports demonstrating an inhibitory effect of metabotropic receptor activation upon stimulation. For example, ACPD has reduced NMDA-induced toxicity in cortical cultures (Ambrosini et al., 1995) and blocked the activation of dopamine release by electrical stimulation (Tabe and Fibiger, 1995) and by K^+-stimulation in the striatum (Verma and Moghaddam, 1998). This indicates that during hyperexcitation the activation of mGluRs may be a mechanism to reduce excitatory amino acid release, thus counteracting hyperactivity. On the other hand, in concert with taurine release the K^+-stimulated GABA release has also been suppressed by group II and III receptors in rat cortical cultures (Schaffhauser et al., 1998). This reduction in inhibitory amino acid release could be harmful and contribute to excitotoxic damage and neuronal degeneration. The potentiation of K^+-evoked taurine release by DCG IV in the immature hippocampus could be due to the additive effects of membrane depolarization and opening of the NMDA receptor-gated ion channels by DCG IV and may not reflect any actions of mGluRs.

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