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Characteristics of taurine release induced by free radicals in mouse hippocampal slices

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Summary. The release of the inhibitory neuromodulator taurine in the hippocampus is markedly enhanced under various neural cell-damaging conditions, including ischemia and exposure to free radicals. The properties and regulation of the release evoked by a medium containing free radicals was investigated in hippocampal slices from adult (3-month-old) and developing (7-day-old) mice, using a superfusion system. The 'free radical damage' was induced by applying 0.01% H_2O_2. The release of [^{3}H]taurine was in both adult and developing hippocampus partly Ca^{2+}-independent, mediated by Na^+-dependent transporters and probably resulting from disruption of cell membranes and subsequent ion imbalance. The release in developing mice appeared to be more susceptible to regulation than that in adults, the stimulation by free radicals being in the latter already maximal. The release was reduced by adenosine A_1 receptor agonist R(−)N^6-(2-phenylisopropyl)adenosine, which effect was, however, abolished by the antagonist 8-cyclopentyl-1,3-dipropylxanthine only in the immature hippocampus, indicating a receptor-mediated process. Moreover, the evoked taurine release in developing mice was potentiated by the ionotropic glutamate receptor agonists N-methyl-D-aspartate, kainate and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate in a receptor-mediated manner, since the effects were abolished by their respective antagonists. The metabotropic glutamate receptors are of only minor significance in the release, the agonists of group I and II receptors slightly reducing the release. Furthermore, NO may also be involved in this release, the NO-generating compounds hydroxylamine and S-nitroso-N-acetylpenicillamine being able to enhance the free-radical-evoked release. It seems that the free-radical-stimulated release, potentiated by ionotropic glutamate receptor activation and NO production, could constitute part of the neuroprotective properties of taurine, being important particularly in the developing hippocampus and hence preventing excitotoxicity.

Keywords: Hippocampal slices – Developing and adult mice – Taurine release – Free radicals – Glutamate receptors – Adenosine receptors

Abbreviations: AIDA: (RS)-1-aminoindan-1,5-dicarboxylate; AMPA: 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; CGS 21680: 7-trifluoromethyl-4-(4-methyl-1-piperazinyl)pyrrolo[1,2-a]quinoxaline; 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; DMPX: 3,7-dimethyl-1-propargylxanthine; DPCPX: 8-cyclopentyl-1,3-dipropylxanthine; EGLU: (2S)-2-ethylglutamate; HA: hydroxylamine; L-AP4: L(+)-2-amino-4-phosphonobutyrate; L-NNA: N^G-nitro-L-arginine; L-SOP: O-phospho-L-serine; NBQX: 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; NMDA: N-methyl-D-aspartate; R-PIA: R(−)N^6-(2-phenylisopropyl) adenosine; SNAP: S-nitroso-N-acetylpenicillamine; t-ACPD: (±)-1-aminocyclopentane-trans-1,3-dicarboxylate.

Introduction

The inhibitory amino acid taurine has been shown to play a pivotal role in the development of the central nervous system and in the survival of neurons (Sturman, 1993; Aerts and Van Assche, 2002). It protects neural cells from excitotoxicity induced by excitatory amino acids in the hippocampus (French et al., 1986) and cerebellum (Trenkner, 1990). It forestalls harmful metabolic events evoked by ischemia and hypoxia (Schurr et al., 1987) and attenuates Ca^{2+} influx in ischemia (Lehmann et al., 1985). Taurine-containing neurons are also fairly resistant to cerebral ischemia induced by 4-vessel occlusion (Wu et al., 1994). Moreover, taurine has long been known to ameliorate symptoms in epilepsy (Oja and Kontro, 1983a). Recent evidence shows that taurine and taurine analogs attenuate oxidative damage to DNA (Messina and Dawson, 2000). Both K^+ depolarization (Oja and Saransaari, 1992) and exposure to glutamate receptor agonists (Saransaari and Oja, 1991) have been shown to induce release of taurine in brain slices. The release of taurine is likewise markedly enhanced in various cell-damaging conditions, including ischemia and increased free radical production (see Saransaari and Oja, 2000a).

The mechanisms of the neuroprotective effects of taurine are not known but may involve neuromodulation, osmoregulatory and antioxidantant and calcium ion regulatory effects (Saransaari and Oja, 1992; Oja and Saransaari, 1996).

Hypoxia, hypoglycemia, ischemia and free radical production are known to lead to neuronal cell damage and death. During the oxygen and glucose deficit arising from ischemia, oxidative metabolism is replaced by anaerobic glycolysis, leading to inefficient generation of the high-energy phosphate reserves necessary to maintain cellular ionic gradients and other metabolic processes. Under certain conditions, including ischemia and ageing, free radical levels and membrane lipid peroxidation may become abnormally high, damaging the membrane structures of nerve cells (Colton and Gilbert, 1985; Pellmar et al., 1989). Free radicals are also thought to be involved in certain neurological diseases (Halliwell, 1992; Bondy, 1995).

The neuromodulator adenosine inhibits the presynaptic release of neurotransmitters, including glutamate (Fredholm and Dunwiddie, 1988; Heron et al., 1993) and hyperpolarizes postsynaptic neurons in the hippocampus (Greene and Haas, 1991). These findings corroborate the suggestion 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 adenosine 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 in normoxia. Activation of these receptors depresses the release in adults and enhances it in developing mice (Saransaari and Oja, 2000b). In ischemia, the receptors potentiate taurine release only in adults (Saransaari and Oja, 2000b). In addition, the NMDA-stimulated taurine release has been shown to be modulated by adenosine receptors only in the developing hippocampus, the enhancing effect also being mediated by the A_1 receptors (Saransaari and Oja, 2003).

NO modulates the release of various neurotransmitters in the brain, including GABA and glutamate (Ohkuma et al., 1995; Segeith et al., 1995). 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, this activating a Ca^{2+}-dependent enzyme, NO synthase (Bredt et al., 1992). The NO formed may act intercellularly or diffuse out and act extracellularly at the soluble guanylyl cyclase to increase the content of 3′,5′-cyclic guanosine monophosphate (Knowles et al., 1989). We have demonstrated that NO-generating compounds are able to modulate taurine release in both immature and mature hippocampus, suggesting the involvement of NO-mediated processes in taurine release both under normal conditions (Saransaari and Oja, 1999a) and in ischemia (Saransaari and Oja, 2002).

We have recently characterized the mechanisms (Saransaari and Oja, 1997a, 1998a) and regulation by glutamate receptors, adenosine and NO (Saransaari and Oja, 1999b, 2000b, 2002b) of ischemia-evoked taurine release in the developing and adult mouse hippocampus. Now we studied the properties and regulation of taurine release evoked by free radical production in hippocampal slices from both immature and mature mice, using a superfusion system.

Materials and methods

Material

NMRI mice of both sexes, aged 3 months (adult) and 6–8 days, were used throughout. [1,2-^{3}H]Taurine (specific radioactivity 1.07 PBq/mol) was obtained from Amersham International (Bristol, UK). All 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 under O_2 and superfused for 50 min 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.

Superfusion conditions

Neural cell damage, designated as 'free radical damage', was induced by modified experimental conditions: 0.01% of H_2O_2 was added to the medium from 1 h before the efflux experiments until the end of superfusion. H_2O_2 generates hydroxyl free radicals with both intracellular and extracellular iron in the Fenton reaction. Exposure to the 0.01% concentration of H_2O_2, which is higher than the concentrations *in vivo*, decreases in hippocampal slices synaptic efficacy and spike generation by one third and one fourth, respectively (Pelmar, 1995).

Estimation of efflux rate constants

The desaturation curves of labeled taurine from the slices were plotted as a function of time on the basis of the radioactivities remaining in the slices after superfusion and recovered in the collected superfusate fractions

(Kontro and Oja, 1987). The efflux rate constants of taurine for the time intervals of 20 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

Free radical formation induced by 0.01% H_2O_2 considerably potentiated [^{3}H]taurine release from hippocampal slices from both 7-day-old and 3-month-old mice, the effect being more pronounced in the former (Fig. 1). The release evoked by H_2O_2 added at the beginning of superfusion evinced a steadily increasing time course, particularly in the slices from 7-day-old mice (Fig. 1). Potassium stimulation (50 mM K^+) at 30 min was then able to enhance the release only in developing mice (Fig. 2A, B). In the absence of Ca^{2+} the H_2O_2-induced release was significantly ($p<0.01$) increased in developing mice but not in adults. There was no K^+ stimulation at either age (Fig. 2). Similarly, in the absence of Mg^{2+} the H_2O_2-induced release significantly increased ($p<0.01$) in the immature hippocampus, but there was no K^+ stimulation (Fig. 2A). In adults, however, K^+ stimulation still potentiated the release (Fig. 2B). Furthermore, omission of Na^+ enhanced the release at both ages, ($p<0.01$). There was then no subsequent K^+ stimulation (Fig. 3); on the contrary, the release was significantly reduced by K^+. Taurine

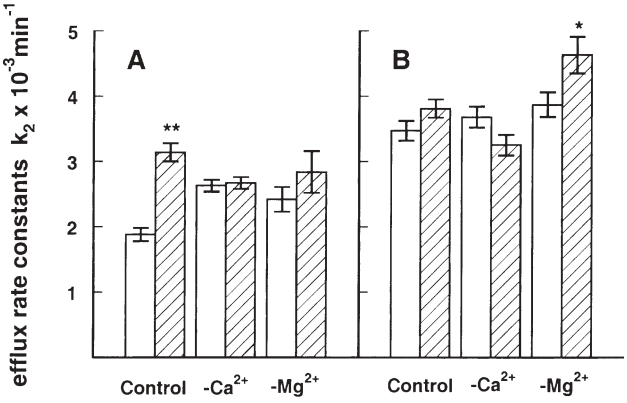

Fig. 2. Effects of omission of Ca^{2+} and Mg^{2+} on taurine release induced by 0.01% H_2O_2 from hippocampal slices from 7-day-old (**A**) and 3-month-old (**B**) mice. Ca^{2+} and Mg^{2+} were omitted from the beginning of the superfusion. The efflux rate constants k_2 (34–50 min) of basal (controls) (open bars) and K^+-stimulated (50 mM K^+ at 30 min) release (hatched bars) are shown. The results are mean values ± SEM of 4–8 independent experiments. Significance of differences from the corresponding control: * $p<0.05$, ** $p<0.01$

(1.0 mM) and glutamate (10 mM) potentiated the free-radical-stimulated release at both ages (Fig. 3), but not in the absence of Na^+ (Fig. 3).

The adenosine A_1 receptor agonist R(−)N^6-(2-phenylisopropyl)adenosine (R-PIA, 0.1 μM) added at the beginning of superfusion depressed the free-radical-stimulated taurine release at both ages (Fig. 1A, B). This effect was abolished by the antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1.0 μM) in the developing hippocampus, but not in the adult (Fig. 1A, B). The adenosine A_{2a}

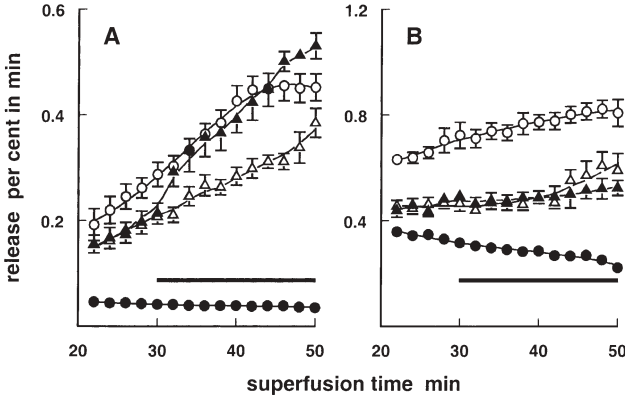

Fig. 1. Time-course of taurine release induced by 0.01% H_2O_2 (-○-) from hippocampal slices from 7-day-old (**A**) and 3-month-old (**B**) mice in the presence of 0.1 μM R(−)N^6-(2-phenylisopropyl)adenosine (-△-) from the beginning of superfusion. The antagonist, 1.0 μM 8-cyclopentyl-1,3-dipropylxanthine was added at 30 min (-▲-), as indicated by the bar. Control basal release without any effectors (-●-). The results are mean values ± SEM of 4–11 independent experiments

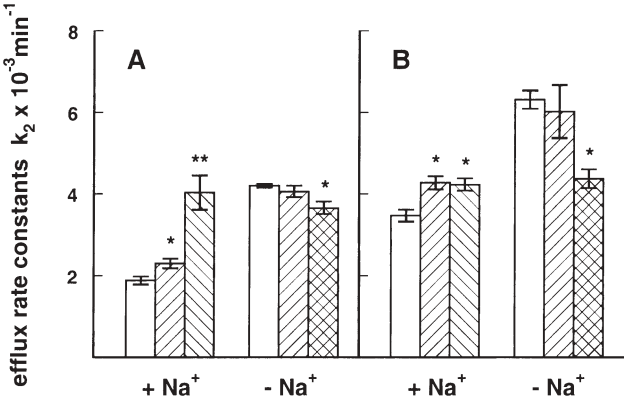

Fig. 3. Taurine release induced by free radical formation (0.01% H_2O_2) in the presence and absence of Na^+ (as indicated) from hippocampal slices from 7-day-old (**A**) and 3-month-old (**B**) mice. The basal release (controls) (open bars), release in the presence of 1.0 mM taurine (right-hatched bars), 10.0 mM glutamate (left-hatched bars) or 50 mM K^+ (cross-hatched bars) applied at 30 min. The results are mean values ± SEM of 4–8 independent experiments. Significance of differences from the corresponding controls (basal releases): * $p<0.05$, ** $p<0.01$

Table 1. Effects of NOergic compounds on taurine release induced by free radicals in mouse hippocampal slices

Compounds present (mM)	Efflux rate constants ± SEM k_2 (34–50 min) × 10^{-3} min^{-1}	
	7-Day-old	3-Month-old
Control	1.88 ± 0.10 (12)	3.47 ± 0.15 (12)
SNAP 1.0	1.82 ± 0.21 (4)	4.80 ± 0.37 ** (4)
SNP 1.0	1.62 ± 0.13 (4)	3.59 ± 0.14 (4)
HA 5.0	4.07 ± 0.43 ** (4)	9.32 ± 1.04 ** (4)
L-NNA 1.0	1.41 ± 0.10 * (4)	2.70 ± 0.14 * (6)

The slices were incubated for 30 min with 10 μM [^{3}H]taurine in Krebs-Ringer-Hepes-glucose medium (pH 7.4) and then superfused for 50 min with 0.01% H_2O_2. NOergic compounds were added at 30 min. Results are rate constants ± SEM for the period of 34–50 min (k_2). Number of independent experiments in parenthesis. Significance of differences from the corresponding controls: * $p < 0.05$, ** $p < 0.01$. *SNAP*, S-nitroso-N-acetylpenicillamine; *SNP*, sodium nitroprusside; *HA*, hydroxylamine; *L-NNA*, N^G-nitro-L-arginine

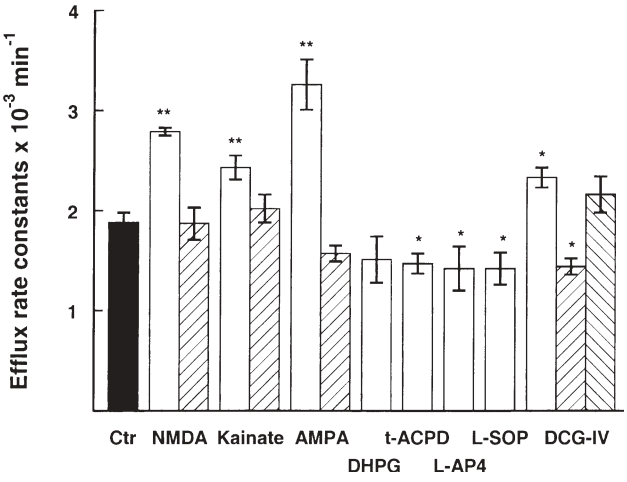

Fig. 4. Effects of glutamatergic compounds on taurine release from hippocampal slices from 7-day-old mice under 'free radical damage', induced by 0.01% H_2O_2 from the beginning of superfusion (control, filled bar). The agonists (indicated at the bottom of the open bars) and the antagonists of NMDA, kainate, AMPA and metabotropic group II receptors, dizocilpine, CNQX, NBQX and EGLU, respectively, (right-hatched bars) and dizocilpine (left-hatched bar) were together applied at 30 min. The concentration of all agonists and antagonists was 0.1 mM. Number of independent experiments 4–8. Significance of differences from the control: * $p < 0.05$, ** $p < 0.01$

receptor agonist 7-trifluoromethyl-4-(4-methyl-1-piperazinyl)pyrrolo[1,2-a]quinoxaline (CGS 21680) (10 μM) also reduced the release ($p < 0.05$), the efflux rate constants k_2 (34–50 min) being $2.68 \pm 0.15 \times 10^{-3}$ min^{-1} (mean ± SEM, $n = 8$) and $1.43 \pm 0.09 \times 10^{-3}$ min^{-1} ($n = 7$) in the adult and developing hippocampus, respectively (cf. Table 1). The antagonist 3,7-dimethyl-1-propargylxanthine (DMPX, 10 μM) had no effect on this reduced release at either age (data not shown).

The evoked taurine release was significantly enhanced by the ionotropic glutamate receptor agonists N-methyl-D-aspartate (NMDA), kainate and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) (all 0.1 mM) in 7-day-old mice (Fig. 4), but not in adults (data not shown). These effects were reduced ($p < 0.01$) by the respective antagonists (all 0.1 mM) dizocilpine, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (Fig. 4). Of the metabotropic glutamate receptor agonists the group I agonist (±)-1-aminocyclopentane-trans-1,3-dicarboxylate (t-ACPD) and the group III agonists, L(+)-2-amino-4-phosphonobutyrate (L-AP4) and O-phospho-L-serine (L-SOP) (all 0.1 mM) significantly reduced taurine release only in developing mice (Fig. 4). Their respective metabotropic glutamate group I and III receptor antagonists (RS)-1-aminoindan-1,5-dicarboxylate (AIDA) and (RS)-2-cyclopropyl-4-phosphonophenylglycine (CPPG) (both 0.1 mM) had no effects on this release (data not shown). The group II agonist (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG IV) enhanced the release. This action was reduced by the antagonist dizocilpine, but not by (2S)-2-ethylglutamate (EGLU) (0.1 mM) (Fig. 4).

The NO generating compounds S-nitroso-N-acetylpenicillamine (SNAP, 1.0 mM) and hydroxylamine (HA, 5 mM) enhanced taurine release evoked by free radical formation in the adult hippocampus, HA being the more potent (Table 1). In the immature hippocampus only HA was effective. The NO synthase inhibitor N^G-nitro-L-arginine (L-NNA, 1.0 mM) inhibited the release at both ages (Table 1).

Discussion

The actions of ischemia and exposure to free radicals share many common features (Gilman et al., 1994), cooperating in the genesis of ischemia-induced neuronal damage. Free radicals cause tissue damage through a multiplicity of mechanisms, including excitotoxicity, metabolic dysfunction and disturbance of the intracellular homeostasis of calcium (Facchinetti et al., 1998). Neural membranes contain a large amount of polyunsaturated fatty acids, which are susceptible to the attack of radicals. Lipid peroxidation may lead to a generalized increase in membrane permeability (Hara et al., 1991) and/or the

activity of membrane constituents, including alterations in Na^+/K^+ ATPases, Ca^{2+} ATPases or the Na^{2+}/Ca^{2+} exchanger (Rohn et al., 1993). Early events in free-radical-mediated damage also produce slow depolarization parallel to an increase in Na^+ and a steady rise in Ca^{2+} (Tretter and Adam-Vizi, 1996). Moreover, exposure to free radicals has been shown to potentiate the release of glutamate and D-aspartate from various brain areas (Pellegrini-Giampietro et al., 1990; Gilman et al., 1994; O'Regan et al., 1997; Saransaari and Oja, 1998b, 1999c). Taurine release was now markedly potentiated in the free-radical-containing media in both immature and mature hippocampus similarly to ischemic conditions (Saransaari and Oja, 2000a). A high concentration of K^+ failed to stimulate the release in adults, suggesting that it was already maximal. A very high concentration of glutamate still evoked taurine release, particularly in the developing hippocampus, which may contribute to prevention of excessive excitotoxicity. This finding is in agreement with the conception that the immature hippocampus is fairly resistant to hypoxic insults (Cherubini et al., 1989; Cherici et al., 1991).

In normoxia, the Ca^{2+}-dependency of taurine release has been greater in the immature hippocampus than in the mature (Saransaari and Oja, 1997a, 1998a, 1999a). In ischemia, the major part has been Ca^{2+}-independent in the adult and aged hippocampus (Saransaari and Oja, 1997a), whereas the attenuation of both basal and K^+-stimulated release in the developing hippocampus in the absence of Ca^{2+} indicates the involvement of Ca^{2+}-dependent processes (Saransaari and Oja, 1999a). There was now no K^+-stimulated release in the free-radical-containing media in adults, and in the immature hippocampus it was only partly Ca^{2+}-dependent. Moreover, the release could also result from excitotoxicity-induced cellular swelling under cell-damaging conditions, which is mainly Ca^{2+}-independent (Sanchez-Olea et al., 1993).

Na^+-free medium is known to diminish the K^+ content of slices (Korpi and Oja, 1983), due to inhibition of Na^+, K^+-ATPase. Both basal and K^+-stimulated hippocampal releases of taurine have been markedly enhanced by Na^+ deficiency in normoxia (Saransaari and Oja, 1998a, 1999a), this bespeaking the involvement of Na^+-dependent taurine transporters operating outwards. Indeed, adult and developing brain tissue possesses saturable, Na^+-requiring transport systems for taurine at neuronal and glial cell membranes, comprising both high- and low-affinity components (Oja and Kontro, 1983b), which could exhibit this kind of behavior. The effects of free radical damage to these transporters is not known, but in the mouse cerebral cortex they are still operative in ischemia, though nonsaturable diffusion is greatly increased (Saransaari and Oja, 1996). The involvement of carriers was now confirmed by homo-trans-stimulation of the release at both ages. Under Na^+-free conditions this stimulation was not discernible, the carriers not operating without Na^+. These results clearly show that a substantial part of the Ca^{2+}-independent taurine release in the presence of free radicals in the hippocampus is mediated by Na^+-dependent transport. On the other hand, the increase in taurine release could result from disruption of Na^+–Ca^{2+} balance caused by peroxide-induced free radicals (Hayashi et al., 1989), resulting in an increase in intracellular Ca^{2+} and enhanced amino acid release. In this situation, K^+-depolarization failed to enhance taurine release, even though the absence of extracellular Na^+ should have potentiated taurine release by the reversal of transporters. In Na^+-free medium, K^+ may even partially adopt the role of Na^+ in promoting the function of transporters, since taurine release from the brain is reduced when an excess of K^+ ions is added to incubation medium (Korpi and Oja, 1983).

Taurine release induced by free radicals was now affected by adenosinergic compounds, both A_1 and A_2 receptor agonists R-PIA and CGS 21680 reducing the release at both ages. However, the inhibition was only receptor-mediated in the immature hippocampus, being abolished by the A_1 antagonist DPCPX. Adenosine A_1 receptor agonists have been shown to protect neuronal cells against ischemic damage *in vivo* by depressing both the basal and the K^+-evoked release of excitatory amino acids (Heron et al., 1994; Goda et al., 1998). Reduced taurine release may thus not be beneficial in this situation. However, in some studies adenosinergic A_1 compounds have not affected glutamate accumulation (Heron et al. 1993) and have suppressed the ischemia-induced GABA release (O'Regan et al., 1992).

The ionotropic glutamate receptor agonists NMDA, kainate and AMPA have concentration-dependently potentiated taurine release in the developing, adult and ageing mouse hippocampus (Saransaari and Oja, 1997a). The stimulations have been markedly greater in the immature than in the adult or ageing hippocampus (Saransaari and Oja, 1997b). The NMDA and AMPA receptors have been shown to be involved in taurine release throughout the whole lifespan of mice, while the kainate-receptor-mediated release does not appear to function in adults (Saransaari and Oja, 1997b). The ability of ionotropic glutamate agonists to evoke taurine release varies under different cell-damaging conditions (Saransaari and Oja, 1997c). Taurine release

evoked by exposure to free radicals was now modulated by glutamate receptors only in the immature hippocampus. All three types of ionotropic glutamate receptors potentiated the release in a receptor-mediated manner, since their respective antagonists were able to abolish the enhancements. The properties and regulation of the NMDA-evoked release of taurine both *in vivo* and *in vitro* is fairly well documented (Saransaari and Oja, 2003). 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). Responses to kainate and AMPA have invariably been greater in slices from the immature than the mature hippocampus both in normal incubation media and under cell-damaging experimental conditions (Saransaari and Oja, 1997b, c), the AMPA effect being now the most potent. The number of kainate and AMPA receptors is relatively small in the rat hippocampus during early postnatal life (Insel et al., 1990; Miller et al., 1990). The strong response of taurine release to these non-NMDA receptor agonists in developing mice is apparently at variance with the relatively low degree of expression of non-NMDA receptors during that period.

Metabotropic glutamate group I and III receptors again appear to be involved in taurine release induced by free radicals in the hippocampus of developing mice, since the receptor agonists trans-ACPD (group I) and L-AP4 and L-SOP (group III) were able to reduce the release, although their antagonists did not now block their effects. Moreover, in comparison to the effects of ionotropic glutamate receptor agonists their effects were rather small. Presynaptic metabotropic glutamate receptors 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., 1996). Group I receptors are known generally to increase neuronal excitation and excitability (see Nicoletti et al., 1996) and to synergize with NMDA receptors in inducing neuronal damage (Sacaan and Schoepp, 1992). The activation of metabotropic group III receptors by L-AP4 and L-SOP also produces neuroprotective effects in cultured neurons and brain slices (Bruno et al., 1995). The ischemia-induced taurine release has generally been unaffected by the metabotropic glutamate receptors in the immature hippocampus, with the exception of potentiation by group I agonists (Saransaari and Oja, 2000c). The marked stimulation of taurine release by DCG IV, a group II agonist, has been shown not to be mediated by the group II receptors, but by ionotropic glutamate receptors in the immature hippocampus in normoxia (Saransaari and Oja, 1999b). The free-radical-evoked release is also reduced by the NMDA receptor antagonist dizocilpine and not by CPPG. It is known that DCG IV behaves as an NMDA receptor agonist at concentrations higher than 10 μM (Uyama et al., 1997).

Free-radical production stimulated taurine release differently from ischemia. The release was in both adult and developing hippocampus partly Ca^{2+}-independent, mediated by Na^{+}-dependent transporters and probably resulting from disruption of cell membranes and subsequent ion imbalance. The release in developing mice appeared to be more susceptible to regulation than that in adults. In the latter the stimulation by free radicals was already maximal. The release in the immature hippocampus was reduced by adenosine A_1 receptor activation and potentiated by ionotropic glutamate receptor agonists in a receptor-mediated manner. The metabotropic glutamate receptors would appear to have only a minor role in the release. Apparently NO is also involved in the taurine release induced by free radicals, since on one hand the NO synthase inhibitor nitroarginine inhibited the release, and on the other the NO generators enhanced it at both ages. Together with the potentiation by NMDA receptors and non-NMDA-receptors, the free-radical-evoked taurine release apparently contributes to protection against excitotoxic neuronal damage, particularly in the immature hippocampus.

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