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Characteristics of taurine release in slices from adult and developing mouse brain stem

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Summary. Taurine has been thought to function as a regulator of neuronal activity, neuromodulator and osmoregulator. Moreover, it is essential for the development and survival of neural cells and protects them under cell-damaging conditions. Taurine is also involved in many vital functions regulated by the brain stem, including cardiovascular control and arterial blood pressure. The release of taurine has been studied both in vivo and in vitro in higher brain areas, whereas the mechanisms of release have not been systematically characterized in the brain stem. The properties of release of preloaded [^{3}H]taurine were now characterized in slices prepared from the mouse brain stem from developing (7-day-old) and young adult (3-month-old) mice, using a superfusion system. In general, taurine release was found to be similar to that in other brain areas, consisting of both Ca^{2+}-dependent and Ca^{2+}-independent components. Moreover, the release was mediated by Na^+-, Cl^--dependent transporters operating outwards, as both Na^+-free and Cl^--free conditions greatly enhanced it. Cl^- channel antagonists and a Cl^- transport inhibitor reduced the release at both ages, indicating that a part of the release occurs through ion channels. Protein kinases appeared not to be involved in taurine release in the brain stem, since substances affecting the activity of protein kinase C or tyrosine kinase had no significant effects. The release was modulated by cAMP second messenger systems and phospholipases at both ages. Furthermore, the metabotropic glutamate receptor agonists likewise suppressed the K^+-stimulated release at both ages. In the immature brain stem, the ionotropic glutamate receptor agonists N-methyl-D-aspartate (NMDA) and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) potentiated taurine release in a receptor-mediated manner. This could constitute an important mechanism against excitotoxicity, protecting the brain stem under cell-damaging conditions.

Keywords: Taurine release – Ca^{2+} dependency – Na^+ and Cl^- ions – Ion channels – Second messengers – Glutamate receptors – Brain stem slices – Adult and developing mice

Abbreviations: 9-AC, 9-antracenecarboxylate; AIDA, (RS)-1-aminoindan-1,5-dicarboxylate; AMPA, 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; 2R,4R-APDC, (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate; cAMP, cyclic adenosine monophosphate; ATPase, adenosine triphosphatase; cGMP, cyclic guanosine monophosphate; DHPG, (S)-3,5-dihydroxyphenylglycine; DIDS, diisothiocyanostilbene-2,2′-disulphonate; EDTA, ethylenediaminetetra-acetate; EGLU, (2S)-2-cyclopropyl-4-phosphonophenylglycine; GABA, γ-aminobutyrate; GAT, GABA transporter; Hepes, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; IBMX, 3-isobutyl-1-methylxanthine; 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; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; NMDA, N-methyl-D-aspartate; ODQ, 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one; PDE, phosphodiesterase; PMA, 4β-phorbol 12-myristate 13-acetate; PKC, protein kinase C; RO 20-1724, 4-(3-butoxy-4-methoxyphenyl)-2-imidazolidone; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonate; trans-ACPD, (±)-1-aminocyclopentane-trans-1,3-dicarboxylate

Introduction

The inhibitory amino acid taurine has been thought to function as a regulator of neuronal activity, inducing hyperpolarization and inhibiting firing of central neurons (Oja and Kontro, 1983a; Saransaari and Oja, 1992). It is also involved in osmoregulation and cell volume adjustments in the central nervous system (Huxtable, 1992; Oja and Saransaari, 1996). Furthermore, taurine has a special role in immature brain tissue (Oja and Kontro, 1983a; Kontro and Oja, 1987; Sturman, 1993). It seems to be essential for the development and survival of neural cells (see Huxtable, 1992; Sturman, 1993). It is also one of the most abundant free amino acids in the brain, and during ontogenic development the concentration of taurine in the central nervous system even exceeds that of glutamate (Oja and Kontro, 1983a). Moreover, taurine protects neural cells from excitotoxicity induced by excitatory amino acids in the hippocampus (French et al., 1986), cerebellum (Trenkner, 1990) and neuronal cultures (Tang et al., 1996), prevents harmful metabolic sequences evoked by ischemia or hypoxia (Schurr et al., 1987), and alleviates symptoms in epilepsy

(Oja and Kontro, 1983b). Various cell-damaging conditions induce a substantial release of taurine in the hippocampus, ischemia being particularly effective (Saransaari and Oja, 1997a, 1998, 1999a), thus providing a protective mechanism against excitotoxicity (Saransaari and Oja, 2000).

The brain stem is the pivotal region in the regulation of multiple sensory and visceral processes, being also the location of the cardiovascular and respiratory centres. The levels of taurine in the brain stem are not so high as in the higher brain regions, but the lateral geniculate nucleus, inferior colliculus and auditory brain stem contain high amounts of taurine, for example (see Oja and Kontro, 1983a; Palkovits et al., 1986). Moreover, the magnitude of postnatal decrease in taurine concentration is several times greater in the medulla than in the cerebral cortex, for example (Cutler and Dudzinski, 1974). Taurine has been suggested to be involved in the ventrolateral medulla in the modulation of cardiovascular control (Kubo et al., 1993; Wang et al., 2005) and in the nucleus of the solitary tract in the neurogenic control of arterial pressure (Meeley et al., 1989). Taurine is released from the locus coeruleus by various stimuli (Singewald and Philippu, 1998) and there is also evidence that the released taurine in the locus coeruleus is involved in conditioned fear (Kaehler et al., 2000). The release processes of taurine have been studied both in vivo and in vitro in higher brain areas, including the hippocampus and striatum (Saransaari and Oja, 1992, 1997a, 1998, 1999a; Phillis and O'Regan, 2003; Molchanova et al., 2004, 2005), but not systematically in the brain stem. Now we wanted to characterize the release mechanisms of preloaded [^{3}H]taurine in slices prepared from the mouse brain stem from developing (7-day-old) and young adult (3-month-old) mice, using a superfusion system. Furthermore, the possible involvement of second messenger systems in the release and regulation by glutamatergic terminals were also addressed.

Materials and methods

Materials

Developing (7-day-old) and young adult (3-month-old) NMRI mice of both sexes were used in the experiments. [^{3}H]Taurine (specific radioactivity 1.07 PBq/mol) was obtained from Amersham International, Bristol, UK. The ion channel inhibitors were from Sigma (St. Louis, MO), except for 9-antracenecarboxylate (9-AC), which was together with other special reagents from Tocris Bioscience (Northpoint, Avonmouth, UK). Other reagents and drugs were from common commercial sources.

Release experiments

Superficial slices 0.4 mm thick weighing 15–20 mg were manually prepared from the mouse brain stem with a tissue slicer of Stadie-Riggs type. The slices were immediately immersed in 5 ml of oxygenated medium and incubated with 0.01 mM [^{3}H]taurine (50 MBq/l) at 37°C for 15 min under agitation. The standard medium contained (in mmol/l) NaCl 127, KCl 5, $CaCl_2$ 0.8, $MgSO_4$ 1.3, Na_2HPO_4 1.3, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (Hepes) 15, NaOH 11 and D-glucose 10 (pH 7.4). The slices were then transferred into 0.25 ml cups and superfused with the 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). The superfusion medium was pooled during the first 20 min and thereafter 2 min fractions (0.5 ml) were directly collected into small scintillation vials with a fraction collector. After superfusion the slices were weighed, homogenized in ice-cold 5% (w/v) trichloracetic acid solution, and centrifuged, and the clear supernatants were used for scintillation counting. The effluent samples were subjected to the same analyses.

Superfusion conditions

In Na^+-free media NaCl was equimolarly substituted by choline chloride added at the beginning of superfusion. In Ca^{2+}-free media Ca^{2+} (0.8 mM) was omitted and ethylenediaminetetra-acetate (EDTA, 2.0 mM) added at the beginning of the superfusion experiments.

Estimation of efflux rate constants

Desaturation curves of labeled taurine from the slices were plotted as a function of time on the basis of the radioactivities remaining in the slices after superfusion and recovered in the collected superfusate fractions (Kontro and Oja, 1987). The efflux rate constants of taurine for the time intervals of 20–30 min (k_1) and 32–40 min (k_2) were computed as negative slopes for the regression lines of the logarithm of radioactivity remaining in the slices vs. superfusion time. The efflux rate constant k_1 denotes the basal prestimulated release and the second efflux rate constant k_2 the K^+-stimulated release.

Statistical calculations

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

Results

The basal unstimulated release of [^{3}H]taurine from brain stem slices was rather steady in 7-day-old mice during the whole experimental time (Fig. 1A), whereas the release from 3-month-olds declined more rapidly (Fig. 1B). Depolarization by K^+ ions (50 mM) between 32 and 40 min enhanced the release almost 4-fold in developing mice, whereas the increase was only 1.3-fold in adults (Fig. 1, Table 1). Characteristically, the evoked release increased in magnitude in developing mice during the whole 20 min stimulation period up to 50 min. When Ca^{2+} was omitted from the superfusion medium, the basal taurine release was greatly enhanced in both age groups ($p < 0.01$), but no significant K^+-stimulation was discernible (Fig. 2).

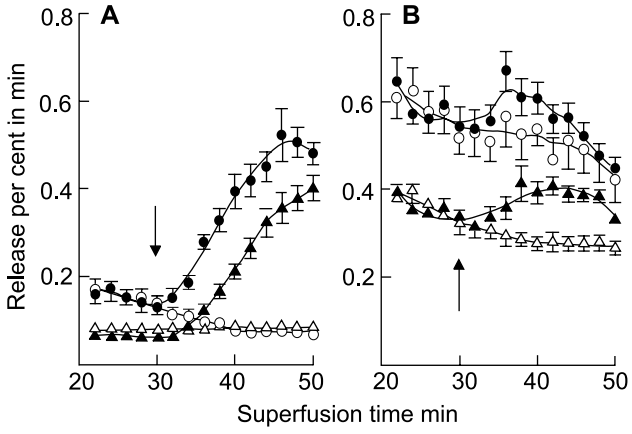

Fig. 1. Time-course of basal (-○-) and K^+-stimulated (-●-) taurine release from brain stem slices from 7-day-old (**A**) and 3-month-old (**B**) mice. The effect of 0.1 mM 3-isobutyl-1-methylxanthine (IBMX) on the basal (-△-) and K^+-stimulated (-▲-) release is also shown. K^+ (50 mM) was added at 30 min as shown by the arrow. The results are means of 4–8 independent experiments with SEMs indicated

When Cl^- was omitted from the superfusion medium the release was considerably ($p < 0.01$) increased in both age groups (Fig. 3). Na^+ omission also potentiated taurine release (Fig. 3). γ-Aminobutyrate (GABA) and taurine (both 1.0 mM) enhanced the release greatly in adults ($p < 0.01$) (Fig. 3B), whereas the potentiation was smaller but nevertheless significant ($p < 0.05$) in developing mice (Fig. 3A). GABA and taurine were not stimulatory under Na^+-free conditions (Fig. 3).

The effects of various ion channel blockers were investigated by adding them to the superfusion medium at the beginning of the release experiments and the subsequent K^+-stimulation was between 30 and 50 min (Table 1). The Na^+ channel blocker riluzole (0.1 mM) had no effect on taurine release in 3-month-olds, but enhanced the basal release and attenuated the K^+-stimulated release in 7-day-olds. The Cl^- channel blockers diisothiocyanostilbene-2,2′-disulphonate (DIDS, 0.5 mM) and 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonate (SITS, 2.0 mM) reduced the initial basal prestimulated release (k_1 constants) and K^+-evoked release (k_2 constants) in both age groups. Moreover, the Cl^- transport inhibitor 9-AC (0.2 mM) also decreased the K^+-stimulated release at both ages.

The effects of several compounds affecting the second messenger systems were investigated by superfusing the slices with these compounds from the beginning and during the subsequent K^+-stimulation up to 50 min (Table 2). The tyrosine kinase inhibitor genistein (1 μM) had no effect on taurine release in adult mice, whereas the K^+-stimulated release in immature mice was significantly diminished. The phospholipase inhibitor quinacrine (0.1 mM) reduced the basal and K^+-evoked release in both age groups. Alloxan, an adenylyl cyclase inhibitor,

Table 1. Effects of ion channel inhibitors on taurine release from brain stem slices from 3-month and 7-day-old mice

Effector	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM			
	3-Month-old		7-Day-old	
	k_1	k_2	k_1	k_2
Basal (control)	2.46 ± 0.06 (76)	2.02 ± 0.08 (7)	0.56 ± 0.03 (47)	0.35 ± 0.05 (8)
+ 50 mM K^+ (control)		2.66 ± 0.09 (7)		1.30 ± 0.09 (8)
Riluzole, 0.1 mM	2.65 ± 0.10 (14)	2.33 ± 0.27 (7)	0.58 ± 0.05 (18)	0.53 ± 0.07** (8)
+ 50 mM K^+		2.73 ± 0.10 (7)		1.01 ± 0.10** (10)
DIDS, 0.5 mM	1.71 ± 0.08** (15)	1.30 ± 0.12**(7)	0.42 ± 0.04** (4)	0.39 ± 0.08 (4)
+ 50 mM K^+		1.76 ± 0.16** (7)		0.57 ± 0.02** (4)
SITS, 2.0 mM	1.55 ± 0.18** (8)	1.22 ± 0.17** (8)	0.22 ± 0.01** (4)	0.21 ± 0.01** (4)
+ 50 mM K^+		1.57 ± 0.07** (8)		0.48 ± 0.04** (4)
9-AC, 0.2 mM	2.17 ± 0.14 (8)	1.98 ± 0.16 (8)	0.53 ± 0.05 (8)	0.58 ± 0.10 (4)
+ 50 mM K^+		2.09 ± 0.13** (8)		1.02 ± 0.17** (4)

All drugs were added at the beginning and were present during the whole superfusion period. Fifty mM K^+ was added at 30 min to every second sample until the end of experiments. The results shown are efflux rate constants ± SEM ($\times 10^{-3}$ min^{-1}) for the time intervals of 20–30 min (basal release, k_1) and 32–40 min (K^+-stimulated release, k_2) with the number of independent experiments in parenthesis. K^+ stimulation significantly ($p < 0.01$) enhanced the release in both age groups. Otherwise, significance of differences from the corresponding controls is the following: * $p < 0.05$, ** $p < 0.01$

DIDS, diisothiocyanostilbene-2,2′-disulphonate; *SITS*, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonate; *9-AC*, 9-anthracenecarboxylic acid

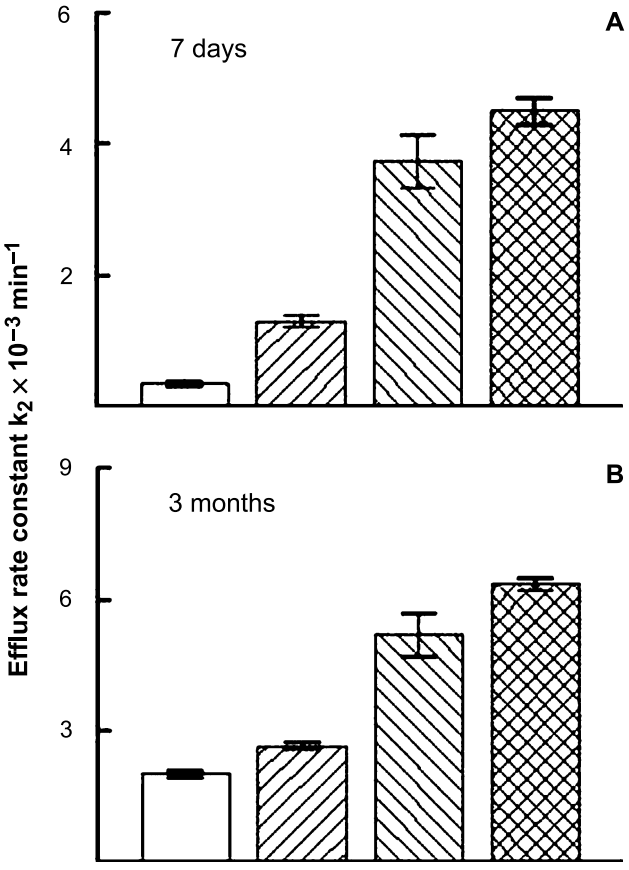

Fig. 2. Effect of Ca^{2+}-free medium on the efflux rate constants k_2 (32–40 min) of taurine release from brain stem slices from 7-day-old (**A**) and 3-month-old (**B**) mice. The first open bars show the basal and the second right-hatched bars the K^+-stimulated release in Ca^{2+}-containing media, the third left-hatched bars the basal and the fourth cross-hatched bars the K^+-stimulated release in Ca^{2+}-free media. The results are mean values ± SEM of 4–8 independent experiments

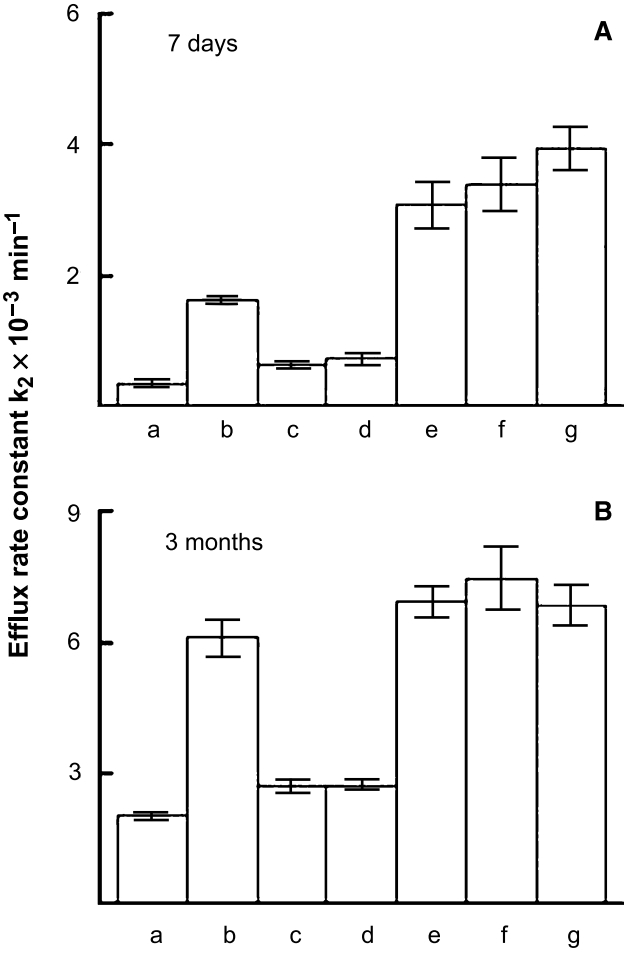

Fig. 3. Effects of Cl^- and Na^+ omission and structural analogues on taurine release from brain stem slices from 7-day-old (**A**) and 3-month-old (**B**) mice. The bars show the efflux rate constants k_2 (32–40 min) as follows: *a* normal conditions (control), *b* Cl^--free medium, *c* 1.0 mM GABA added at 30 min in normal medium, *d* 1.0 mM taurine added at 30 min in normal, *e* Na^+-free medium, *f* 1.0 mM GABA added at 30 min in Na^+-free medium, *g* 1.0 mM taurine added at 30 min in Na^+-free medium. The results are mean values ± SEM of 4–8 independent experiments

reduced the K^+-evoked release only in 7-day-old mice (Table 2). The unspecific phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine (IBMX, 1.0 mM) and the selective PDE4 inhibitor 4-(3-butoxy-4-methoxyphenyl)-2-imidazolidone (RO 20-1724, 0.2 mM) markedly diminished the basal release in developing mice, but did not affect the relative magnitude and time course of the subsequent K^+ stimulation (Fig. 1, Table 2). Only IBMX exhibited similar but less pronounced effects in adults. Another PDE inhibitor zaprinast, selective for PDE5, 6 and 9, abolished the K^+-evoked release in 3-month-old mice (Table 2). On the other hand, the inhibitor of soluble guanylate cyclase 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one (ODQ, 0.01 mM) had no effects. Neither did the activator of protein kinase C 4β-phorbol 12-myristate 13-acetate (PMA, 10 nM) and the inhibitor chelerythrine (1 μM).

Glutamate (1.0 mM) had no effect on basal brain stem taurine release in adults (data not shown), but in immature mice the release was potentiated (Fig. 4). The ionotropic receptor agonists kainate, N-methyl-D-aspartate (NMDA) and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) (all 0.1 mM) did not affect the basal or K^+-stimulated release, neither when added to the superfusion medium at 30 min nor at the beginning of the efflux experiments (data not shown). On the other hand, in the immature brain stem NMDA and AMPA enhanced the basal release when added both at the beginning (data not shown) and at 30 min (Fig. 4). The effect of NMDA was abolished by the antagonist dizocilpine

Table 2. Effects of compounds involved in the second messenger systems on taurine release from brain stem slices from 3-month-old and 7-day-old mice

Effector	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM			
	3-Month-old		7-Day-old	
	k_1	k_2	k_1	k_2
Basal (control)	2.46 ± 0.06 (76)	2.02 ± 0.08 (7)	0.56 ± 0.03 (47)	0.35 ± 0.05 (8)
+ 50 mM K^+ (control)		2.66 ± 0.09 (72)		1.30 ± 0.09 (8)
Genistein, 0.001 mM	2.19 ± 0.08 (14)	1.87 ± 0.10 (7)	0.45 ± 0.03 (8)	0.44 ± 0.05 (4)
+ 50 mM K^+		2.26 ± 0.09 (7)		1.14 ± 0.06* (4)
Quinacrine, 0.1 mM	1.44 ± 0.05** (15)	1.27 ± 0.06** (8)	0.32 ± 0.02** (8)	0.37 ± 0.05 (4)
+ 50 mM K^+		1.33 ± 0.07** (7)		0.54 ± 0.04** (4)
PMA, 0.00001 mM	2.42 ± 0.11 (7)	2.23 ± 0.19 (4)	0.58 ± 0.03 (8)	0.57 ± 0.07 (4)
+ 50 mM K^+		2.45 ± 0.23 (4)		1.44 ± 0.10 (4)
Chelerythrine, 0.001 mM	2.89 ± 0.13 (8)	2.53 ± 0.13 (4)	0.50 ± 0.02 (8)	0.43 ± 0.02 (4)
+ 50 mM K^+		2.83 ± 0.24 (4)		1.21 ± 0.10 (4)
IBMX, 1.0 mM	1.67 ± 0.05** (8)	1.26 ± 0.08** (4)	0.30 ± 0.04** (7)	0.36 ± 0.10 (4)
+ 50 mM K^+		1.85 ± 0.12** (4)		0.64 ± 0.06** (4)
RO 20-1724, 0.2 mM	2.34 ± 0.08 (12)	2.20 ± 0.08 (8)	0.31 ± 0.03** (8)	0.33 ± 0.01 (4)
+ 50 mM K^+		2.66 ± 0.11 (4)		0.75 ± 0.10** (4)
Zaprinast, 0.1 mM	2.36 ± 0.24 (6)	2.07 ± 0.31 (4)	0.59 ± 0.07 (8)	0.46 ± 0.07 (4)
+ 50 mM K^+		2.16 ± 0.03** (4)		1.36 ± 0.19 (4)
ODQ, 0.01 mM	2.62 ± 0.09 (8)	2.35 ± 0.13 (4)	0.55 ± 0.02 (8)	047 ± 0.03 (4)
+ 50 mM K^+		2.58 ± 0.16 (4)		1.28 ± 0.08 (4)
Alloxan, 5.0 mM	2.21 ± 0.14 (8)	1.64 ± 0.18 (4)	0.44 ± 0.05 (8)	0.43 ± 0.06 (4)
+ 50 mM K^+		2.59 ± 0.09 (4)		0.76 ± 0.15** (4)

The drugs were added at the beginning of the superfusion and 50 mM K^+ at 30 min as in Table 1. The results show the efflux rate constants ± SEM ($\times 10^{-3}$ min^{-1}) for the time intervals of 20–30 min (k_1) and 32–40 min (k_2) with the number of independent experiments in parenthesis. Significance of differences from the corresponding controls: * $p < 0.05$, ** $p < 0.01$
IBMX, 3-isobutyl-1-methylxanthine; *ODQ*, 1H-(1,2,4)oxadiazolo(4,3-a)guinoxalin-1-one, *IBMX*, 3-isobutyl-1-methylxanthine, *PMA*, 4β-phorbol 12-myristate 13-acetate

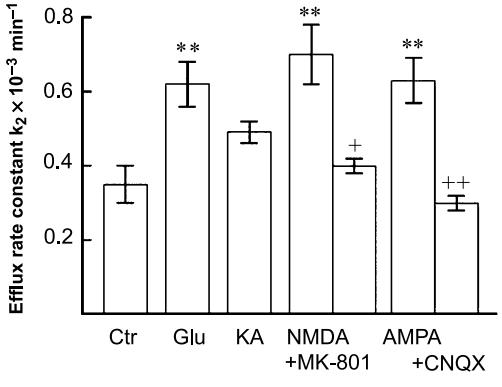

Fig. 4. Effects of ionotropic glutamate receptor agonists and antagonists on taurine release from brain stem slices from 7-day-old mice. The compounds were added at 30 min at the concentration of 0.1 mM, except for glutamate 1.0 mM. The bars show mean values (±SEM of 4–8 independent experiments) of the efflux rate constants k_2 (32–40 min). Significance of differences from the control: $^{**}p < 0.01$. The effect of NMDA was significantly ($^+p < 0.05$) reduced by MK-801 and that of AMPA by CNQX ($^{++}p < 0.01$). *NMDA*, N-methyl-D-aspartate, *KA*, kainate, *AMPA*, 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate, *CNQX*, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide

[(5S, 10R)-(+)-methyl-10,11-dihydro-5*H*-dibenzo(a,d) cyclohepten-5,10-amine, MK-801] and that of AMPA by its antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f] quinoxaline-7-sulfonamide (NBQX 0.1, mM) (Fig. 4). Furthermore, of the agonists of metabotropic glutamate receptors (±)-1-aminocyclopentane-trans-1,3-dicarboxylate (trans-ACPD, group I) inhibited K^+ stimulation at both ages, but these effects were not affected by the antagonist (RS)-1-aminoindan-1,5-dicarboxylate (AIDA, 0.1 mM) (Table 3). Another group I agonist (S)-3,5-dihydroxyphenylglycine (DHPG, 0.1 mM) had no effects on the release. The agonist (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (2R,4R-APDC, 0.1 mM, group II) reduced the stimulated release in adults, which effect was abolished ($p < 0.01$) by the antagonist (2S)-2-cyclopropyl-4-phosphonophenylglycine (EGLU, 0.1 mM) in adults but not in developing mice. Moreover, the release was unaffected by the agonist O-phospho-L-serine (L-SOP) (0.1 mM, group III) at both ages, while the agonist L(+)-2-amino-4-phosphonobutyrate (L-AP4) (0.1 mM, group III) inhibited the K^+-stimulated release in immature mice (Table 3).

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

Concentration (mM)	Efflux rate constants ($\times 10^{-3}$ min^{-1}) ± SEM			
	3-Month-old		7-Day-old	
	k_1	k_2	k_1	k_2
Basal (control)	2.46 ± 0.06 (76)	2.02 ± 0.08 (7)	0.56 ± 0.03 (47)	0.35 ± 0.05 (8)
+ 50 mM K^+ (control)		2.66 ± 0.09 (72)		1.30 ± 0.09 (8)
t-ACPD 0.1	2.24 ± 0.14 (14)	1.97 ± 0.07 (4)	0.55 ± 0.04 (15)	0.42 ± 0.02 (4)
+ 50 mM K^+		2.20 ± 0.06** (4)		1.05 ± 0.06** (5)
+ 50 mM K^+ + AIDA 0.1		2.41 ± 0.18 (4)		1.13 ± 0.06 (4)
DHPG 0.1	2.62 ± 0.11 (7)	2.07 ± 0.10 (4)	0.53 ± 0.02 (8)	0.43 ± 0.02 (4)
+ 50 mM K^+		2.53 ± 0.24 (4)		1.23 ± 0.03 (4)
2R,4R-APDC	2.43 ± 0.10 (13)	2.28 ± 0.16 (4)	0.57 ± 0.05 (11)	0.49 ± 0.08 (4)
+ 50 mM K^+		2.26 ± 0.06** (4)		1.10 ± 0.14 (4)
+ 50 mM K^+ + EGLU 0.1		2.68 ± 0.06 (6)		1.14 ± 0.13 (4)
L-SOP 0.1	2.60 ± 0.11 (11)	2.25 ± 0.20 (7)	0.57 ± 0.06 (10)	0.46 ± 0.05 (7)
+ 50 mM K^+		2.75 ± 0.13 (4)		1.54 ± 0.41 (4)
L-AP4 0.1	2.45 ± 0.12 (8)	2.33 ± 0.20 (4)	0.45 ± 0.02 (15)	0.38 ± 0.02 (6)
+ 50 mM K^+		3.03 ± 0.38 (4)		0.82 ± 0.08** (4)
+ 50 mM K^+ + CPPG 0.1		–		1.05 ± 0.06 (4)

The compounds were added at the beginning of superfusion and 50 mM K^+ at 30 min. The results show the efflux rate constants ± SEM ($\times 10^{-3}$ min^{-1}) for the time intervals of 20–30 min (k_1) and 32–40 min (k_2) with the number of independent experiments in parenthesis. Significance of differences from the corresponding controls: * $p < 0.05$, ** $p < 0.01$

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; *CPPG* (RS)-2-cyclopropyl-4-phosphonophenylglycine; *EGLU* (2S)-2-cyclopropyl-4-phosphonophenylglycine; *L-AP4* L(+)-2-amino-4-phosphonobutyrate; *L-SOP* O-phospho-L-serine

Discussion

The general properties of taurine release in both adult and developing brain stem appeared to be similar to those in the hippocampus (Saransaari and Oja, 1998, 1999b). Depolarization by K^+-stimulation was able to potentiate taurine release at both ages. In developing mice, the response was about only one half of that in the hippocampus (Saransaari and Oja, 1999a). The evoked release of taurine from nervous tissue generally occurs by two distinct main mechanisms, one requiring the entry of Ca^{2+} during depolarization and involving exocytosis, and the other also being due to depolarization but Ca^{2+}-independent in nature (Saransaari and Oja, 1992). Furthermore, there may occur other Ca^{2+}-independent release by several mechanisms, as discussed later. As in the hippocampus, both these main components were also now discernible in the brain stem at both ages. The absence of K^+ stimulation in the absence of Ca^{2+} indicates that a part of the release is clearly Ca^{2+}-dependent, characteristic of a neurotransmitter.

Taurine release in the brain stem was strongly stimulated by taurine itself and also by the structural analogue GABA, suggesting that both homo- and heteroexchange are operating in this brain area. Furthermore, the release was markedly affected by Na^+ deficiency, as in other brain regions (see Saransaari and Oja, 1992). Na^+-free medium is known to diminish the K^+ content of the slices (Korpi and Oja, 1983), due to inhibition of Na^+, K^+-ATPase. This bespeaks the involvement of Na^+-dependent taurine transporters operating outwards. Indeed, brain tissue possesses a saturable, Na^+-requiring transport system for taurine at neuronal and glial cell membranes, comprising both high- and low-affinity components (Oja and Kontro, 1984; Holopainen et al., 1987). The cloned taurine transporter has been localized in the brain stem and pontine fibers in the mouse (Liu et al., 1992) and could exhibit this kind of behavior. Furthermore, taurine is able to interact with GABA transporters (GAT, Palacín et al., 1998; Sivakami et al., 1992). GAT 1, 2 and 3 are all expressed in the brain stem on neuronal and glial membranes (López-Corcuera et al., 1992; Palacín et al., 1998). The taurine transporter also exhibits profound Cl^--dependence (Smith et al., 1992). When the Na^+-gradient is dissipated, the preferred direction of transport changes from inward (uptake) to outward (release). The involvement of transporters in the release in both immature and adult mice was further

confirmed by the effects of taurine and GABA. Under Na^+-free conditions the stimulation by these amino acids was not discernible, the carriers not being functional without Na^+. Furthermore, the chloride channel antagonist DIDS and SITS as well as the chloride transport inhibitor 9-AC reduced the release, indicating that it may occur through anion channels, similarly to the volume-sensitive taurine release in astrocytes and neurons, for example (Schousboe et al., 1991).

Activation of presynaptic protein kinase C (PKC) has enhanced the release of neurotransmitters from nerve terminals (Barrie et al., 1991; Eboli et al., 1993) and protein kinases/phosphatases may also play a role in cell volume regulation (Grinstein et al., 1992). PKC has enhanced taurine release in mouse hippocampal slices (Saransaari and Oja, 2002) and in the rat cortex in vivo during hyposmotic stress (Estevez et al., 1999), whereas 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 PKC activator PMA or inhibitor chelerythrine had now no effects on taurine release in the brain stem, which agrees with results in glial cells from the rat supraopticus nuclei (Deleuze et al., 2000). Tyrosine kinases seem to have only a small effect on release in the brain stem, since the inhibitor genistein reduced the stimulated efflux only in immature mice. However, the involvement of tyrosine kinases in taurine release induced by the hyposmotic stress has been thought to obtain in various cell types, including brain cells, on the basis of the effects of tyrosine kinase and phosphatase blockers (Mongin et al., 1999; Deleuze et al., 2000; Morales-Mulia et al., 2001). The involvement of cyclic guanosine monophosphate (cGMP) in the release in the brain stem seems to be unlikely, although cGMP is able to modulate taurine release in the mouse hippocampus (Saransaari and Oja, 2002), since the inhibitor of soluble guanylate cyclase ODQ had no effects. To date, ODQ has potentiated taurine release in the hippocampus (Saransaari and Oja, 2002). On the other hand, cyclic adenosine monophosphate (cAMP) may be involved because alloxan, an inhibitor of cyclic adenylyl cyclase, suppressed the stimulated release in developing mice. The inhibitory effects of cAMP-selective phosphodiesterase inhibitor RO 20–1724 and the nonselective inhibitors IBMX and zaprinast on the basal and stimulated taurine release at both ages corroborate this assumption. At variance, the cGMP/nitric oxide (NO) pathway modifies the release in the mouse hippocampus (Saransaari and Oja, 2002).

Phospholipase-induced plasma membrane disruption has been suggested to be involved in the ischemia-evoked release of neurotransmitter amino acids in the cerebral cortex (O'Regan et al., 1995; Phillis and O'Regan, 2003). Topical application of phospholipase A_2 has elicited a profound increase in the cortical superfusate levels of taurine (Phillis et al., 1997). Volume-activated taurine release in hippocampal slices (Franco et al., 2001) and in the rat brain in vivo (Estevez et al., 1999) has been insensitive to phospholipase inhibitors. On the other hand, under normal conditions in the developing and adult mouse hippocampus (Saransaari and Oja, 1998, 1999a) the phopholipase A_2 inhibitor quinacrine has potentiated taurine release, indicating that other mechanisms than membrane disruption participate.

Brain stem nuclei serve a diverse array of functions in which ionotropic glutamate receptors are known to be involved (Pierrefiche et al., 1994). The three types on ionotropic receptors are located in the brain stem nuclei (Paarmann et al., 2000; Liu and Wong-Riley, 2004). Ionotropic glutamate receptor agonists now failed to have any effects on taurine release in the adult brain stem. This is at variance with the effects observed in higher brain areas, the cerebral cortex (Saransaari and Oja, 1991) and hippocampus. The ionotropic glutamate receptor agonists NMDA, kainate and AMPA have potentiated concentration-dependently taurine release in the developing, adult and ageing mouse hippocampus (Saransaari and Oja, 1997b). However, the stimulations have been markedly greater in the immature than adult or ageing hippocampus (Saransaari and Oja, 1997b). NMDA and AMPA receptors seem to be involved in taurine release only in the immature brain stem, since the antagonists of NMDA and AMPA receptors were able to abolish their effects. On the other hand, the kainate-receptor mediated release did not appear to function in the brain stem. The release of taurine enhanced by activation of NMDA and AMPA receptors might constitute an important mechanism against excitotoxicity, protecting the brain stem under cell-damaging conditions. Brain stem nuclei also contain metabotropic glutamate receptors (Azcue et al., 1997; de Novellis et al., 2003). The activation of group II and III receptors suppresses the release of excitatory amino acids (Cartmell and Schoepp, 2000), whereas group I receptors enhance glutamate release (Cartmell and Schoepp, 2000) and increase neuronal excitability (Nicoletti et al., 1996). We have shown that activation of all metabotropic glutamate receptors is able to potentiate the basal release of taurine in mouse hippocampal slices, particularly in immature animals (Saransaari and Oja, 1999b). In the brain stem, the regulation seems to be somewhat different, since the metabotropic glutamate receptor agonists did not affect

the basal release. The K^+-stimulated release in the hippocampus has been mainly inhibited by the metabotropic agonists (Saransaari and Oja, 1999b), as now in the brain stem. The suppression of release in adults by the group II agonist 2R,4R-APDC was antagonized by its antagonist EGLU, signifying a receptor-mediated mechanism. The activation of group I receptors by t-ACPD inhibited the stimulated release at both ages, but this effect was not clearly receptor-mediated. The metabotropic glutamate receptor agonists have also suppressed the K^+-stimulated GABA release in the brain stem (Saransaari and Oja, 2005). This reduction in inhibitory amino acid release could be harmful and contribute to excitotoxic damage and neuronal degeneration.

In general, the release of taurine from the brain stem from 3-month-old and 7-day-old mice was found to be similar to that in other brain areas, consisting of both Ca^{2+}-dependent and Ca^{2+}-independent components. Moreover, the release was mediated by Na^+-, Cl^--dependent transporters operating outwards and partly by ion channels. Protein kinases seem not to be involved in taurine release in the brain stem, but the release is modulated by the cAMP second messenger systems and phospholipases at both ages. Furthermore, the metabotropic glutamate receptor agonists likewise suppressed the K^+-stimulated release at both ages. In the immature brain stem, taurine release is potentiated by the ionotropic glutamate receptor agonists NMDA and AMPA, which could constitute an important mechanism against excitotoxicity, protecting the brain stem under cell-damaging conditions.

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