Amino Acids (2000) 18: 17–30

Amino
Acids

Taurine release modified by GABAergic agents in hippocampal slices from adult and developing mice

P. Saransaari[1] and **S. S. Oja**[1,2]

[1]Tampere Brain Research Center, University of Tampere Medical School, Tampere, Finland
[2]Department of Clinical Physiology, Tampere University Hospital, Tampere, Finland

Accepted August 31, 1999

Summary. In order to characterize the possible regulation of taurine release by GABAergic terminals, the effects of several agonists and antagonists of GABA receptors on the basal and K^+-stimulated release of [^{3}H]taurine were investigated in hippocampal slices from adult (3-month-old) and developing (7-day-old) mice using a superfusion system. Taurine release was concentration-dependently potentiated by GABA, which effect was reduced by phaclofen, saclofen and (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA) at both ages, suggesting regulation by both $GABA_B$ and $GABA_C$ receptors. The involvement of $GABA_A$ receptors could not be excluded since the antagonist bicuculline was able to affect both basal and K^+-evoked taurine release. Furthermore, several $GABA_B$ receptor effectors were able to inhibit K^+-stimulated taurine release in the adults, while the $GABA_C$ receptor agonists trans-4-aminocrotonic acid (TACA) and cis-4-aminocrotonic acid (CACA) potentiated this release. The potentiation of taurine release by agents acting on the three types of GABA receptors in both adult and developing hippocampus further indicates the involvement of transporters operating in an outward direction. This inference is corroborated by the moderate but significant inhibition of taurine uptake by the same compounds.

Keywords: Amino acids – Taurine release – GABA receptors – Hippocampal slices – Adult – Developing mouse

Abbreviations: 3-APPA: 3-aminopropylphosphonic acid; CACA: cis-4-aminocrotonic acid; SKF 97541: 3-aminopropyl(methyl)phosphinic acid; TACA: trans-4-aminocrotonic acid; THIP: 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; TPMPA: (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid; ZAPA: (Z)-3-[(aminoiminomethyl)thio]prop-2-enoic acid.

Introduction

The major part of excitatory innervation in the hippocampus, including pyramidal cells, is glutamatergic, the function of these neurons being modulated by inhibitory GABA-releasing interneurons (Frotscher et al., 1984; Freund and Buzsáki, 1988). These GABAergic interneurons in the hippocampus are known to mediate inhibition of feedforward inhibition from the granule cells (Alger and Nicoll, 1982) or of recurrent feedback inhibition from the pyramidal neurons (Andersen et al., 1962). The structural analogue of GABA, taurine, has been held to have a special role in immature brain tissue (Oja and Kontro, 1983; Kontro and Oja, 1987). Besides being an osmoregulator and neuromodulator, this inhibitory amino acid seems to be essential for the development and survival of neural cells (Huxtable, 1992; Sturman, 1993). Taurine abounds in the hippocampus (Lombardini, 1976; Kontro et al., 1980) and taurine-like immunoreactivity has been located in hippocampal interneurons, pyramidal neurons and dentate granule cells (Magnusson et al., 1989). Particularly high concentrations of taurine are found in the immature hippocampus (Saransaari and Oja, 1998). Taurine inhibits the firing of hippocampal pyramidal neurons by increasing the 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), and in hippocampal astrocytes (Reymond et al., 1996).

It has recently been shown that both ionotropic and metabotropic glutamate receptors can modify taurine release in the hippocampus (Magnusson et al., 1991; Saransaari and Oja, 1997; 1999), whereas nothing is known about the regulation of taurine release by GABAergic terminals. In the cerebral cortex and cerebellum GABAergic compounds are known to affect both basal and depolarization-evoked taurine release (Kontro and Oja, 1989; Neal and Shah, 1989). GABA activates three possible classes of receptors. $GABA_A$ receptors are responsible for fast postsynaptic inhibition by the opening of an anion channel (Johnston, 1996a), while $GABA_B$ receptors initiate slower inhibition via G-protein-coupled action on Ca^{2+} and K^+ channels (Misgeld et al., 1995). A third class, tentatively called $GABA_C$ receptor, also mediates its response by means of chloride currents (Bormann and Feigenspan, 1995; Johnston, 1996b). In order to characterize the possible regulation of taurine release by GABAergic terminals, we now studied the effects of several agonists and antagonists of the three GABA receptors on the basal and potassium-stimulated release of [^{3}H]taurine in hippocampal slices from adult and developing 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 changed in many experiments to another modified medium. After superfusion the slices were weighed, homogenized in ice-cold 5% (w/v) trichloracetic acid solution and after centrifugation the clear supernatants used for scintillation counting. The effluent samples were likewise counted for radioactivity.

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.

Uptake experiments

Synaptosomal preparations were obtained from the cerebral cortex of developing and adult mice and incubated in Krebs-Ringer-Hepes-glucose medium (pH 7.4) as described in detail by Kontro (1984). The synaptosomes were first preincubated under O_2 for 10 min and then for 10 min with 30 MBq/l of [^{3}H]taurine. The protein content of the synaptosomes was measured according to Lowry et al. (1951).

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 was concentration-dependently potentiated by GABA, the effect being greater in the immature than in the adult hippocampus (Fig. 1). The GABA effect was not influenced by the $GABA_A$ receptor antagonist bicuculline nor the $GABA_C$ antagonist 3-aminopropyl(methyl)phosphinic acid (SKF 97541), whereas it was reduced by the $GABA_B$ receptor antagonists phaclofen and saclofen in both age groups (Fig. 2). Also (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA) inhibited the GABA effect (Fig. 2). When Na^+ was omitted from the beginning of the superfusion the efflux rate constant k_2 (34–50 min) increased to $4.05 \pm 0.20 \times 10^{-3}\,min^{-1}$ (mean ± SEM, n = 8) and $2.71 \pm 0.27 \times 10^{-3}\,min^{-1}$ (n = 4) in the adult and developing hippocampus, respectively. When 0.1 mM GABA was added to this Na^+-deficient medium, the efflux rate constants were not significantly altered.

Of the compounds affecting $GABA_A$ receptors, trans-4-aminocrotonic acid (TACA) was the most potent in stimulating the basal release of taurine

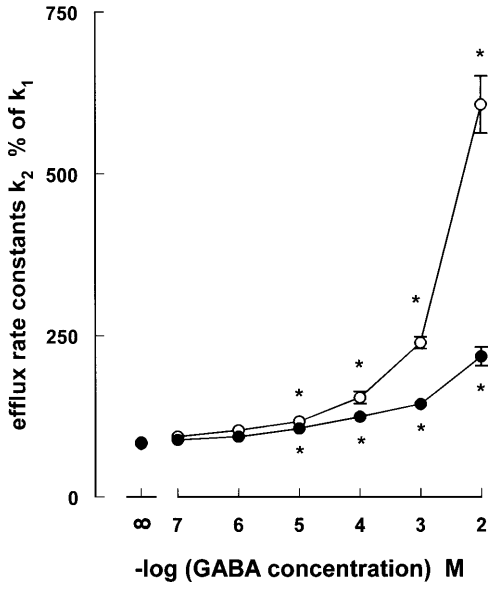

Fig. 1. Stimulation of taurine release in hippocampal slices from 3-month-old (-●-) and 7-day-old (-○-) mice as a function of GABA concentration. GABA was added to the superfusion medium at 30 min. The results are mean values ± SEM of efflux rate constants k_2 (34–50 min) as percentages of k_1 (20–30 min). Number of independent experiments 4–8. Significance of differences from the corresponding controls without GABA: *$p < 0.01$

in the immature hippocampus (Table 1). The effect of TACA (0.1 mM) was unaltered by bicuculline and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyrin-3-ol (THIP) at both ages, whereas (Z)-3-[(aminoiminomethyl)thio]-prop-2-enoic acid (ZAPA), a $GABA_C$ antagonist and agonist at the low-affinity GABA receptor, reduced it only in developing mice ($p < 0.01$) (Table 1). ZAPA alone stimulated taurine release also in the immature hippocampus, which effect was not significantly affected by bicuculline. Moreover, THIP was without effect, but bicuculline alone stimulated the release at both ages. All the tested $GABA_B$ ligands, baclofen, SKF 97541 and 3-aminopropylphosphonic acid (3-APPA) stimulated taurine release, the effects of baclofen and SKF 97541 being somewhat stronger in the developing than in the adult hippocampus (Table 2). The antagonist phaclofen had no effect on the stimulation by baclofen, nor did saclofen influence the action of SKF 97541. The $GABA_C$ receptor compounds cis-4-aminocrotonic acid (CACA) and TACA both potentiated taurine release, the effect of TACA being marked in slices from immature mice (Table 3). The CACA effect was increased by the presence of TPMPA in the adults, but not in developing mice. The TACA effect was not altered by TPMPA at either age (Table 3).

Potassium stimulation (50 mM K^+) enhanced taurine release about 2- and 8-fold in the adult and developing hippocampus, respectively (Table 4). GABA had a weak enhancing effect on this evoked release in immature mice. Moreover, the stimulated release in these animals was not affected by the

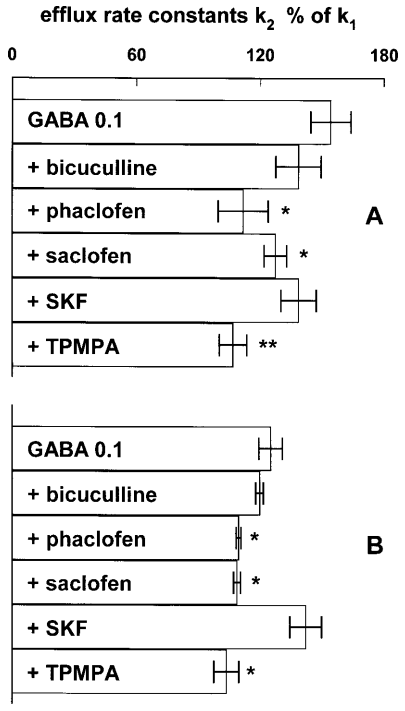

Fig. 2. Effects of antagonists on 0.1 mM GABA-stimulated taurine release in hippocampal slices from 7-day-old (**A**) and 3-month-old (**B**) mice. GABA was added to the superfusion medium together with the antagonists (0.1 mM) at 30 min. The results are mean values ± SEM of efflux rate constants k_2 (34–50 min) as percentages of k_1 (20–30 min). Number of independent experiments 4–8. Significance of differences from the GABA-stimulated release: *$p < 0.05$; **$p < 0.01$. *SKF*, 3-aminopropyl-(methyl)phosphinic acid; *TPMPA*, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid

tested agonists and antagonists. On the other hand, many compounds inhibited this release in the adults: THIP, bicuculline, baclofen, phaclofen, SKF 97541 and TPMPA (all 0.1 mM) all significantly inhibited the K^+-evoked release (Table 4), while TACA and CACA (0.1 mM) enhanced it (Fig. 3). The effects of TACA and CACA were reduced, but not totally abolished by TPMPA (Fig. 3, Table 4).

Most of the tested GABAergic compounds significantly, but not very markedly, inhibited taurine uptake in synaptosomal preparations isolated from the mouse cerebral cortex (Table 5). GABA itself and all agonists and antagonists with the exception of baclofen were effective in the immature hippocampus.

Discussion

GABAergic substances could in principle influence taurine release by several mechanisms. The release could be regulated by presynaptic GABA receptors,

Table 1. Effects of $GABA_A$ compounds on taurine release from mouse hippocampal slices

Compound (mM)	Efflux rate constants k_2 (34–50 min) ± SEM (% of k_1)	
	3-month-old	7-day-old
None (control)	82.9 ± 2.9 (14)	84.7 ± 2.3 (13)
TACA 0.01	89.2 ± 4.7 (4)	99.3 ± 6.6** (7)
TACA 0.1	100.1 ± 5.3* (4)	189.7 ± 29.7** (4)
+bicuculline 0.1	112.7 ± 2.1** (4)	192.3 ± 15.1** (4)
+THIP 0.1	127.4 ± 4.6** (4)	165.9 ± 26.4** (4)
+ZAPA 0.1	111.6 ± 4.7** (4)	92.4 ± 6.2 (8)
ZAPA 0.01	–	109.2 ± 13.2* (8)
ZAPA 0.1	84.1 ± 5.5 (8)	105.5 ± 14.9* (4)
+bicuculline 0.1	107.3 ± 7.2** (4)	95.6 ± 4.1* (4)
THIP 0.1	95.2 ± 5.4 (4)	86.7 ± 5.3 (8)
Bicuculline 0.1	110.3 ± 2.4** (4)	104.3 ± 5.1** (4)

The slices were first 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 the above effectors. The results are percentages of the basal efflux rate constant (k_1) of each slice. Number of independent experiments in parentheses. Significance of differences from the corresponding controls: *$p < 0.05$, **$p < 0.01$. *TACA*, trans-4-aminocrotonic acid; *THIP*, 4,5,6,7-tetrahydro-isoxazolo[5,4-c]pyridin-3-ol; *ZAPA*, (Z)-3-[(aminoiminomethyl)thio]prop-2-enoic acid.

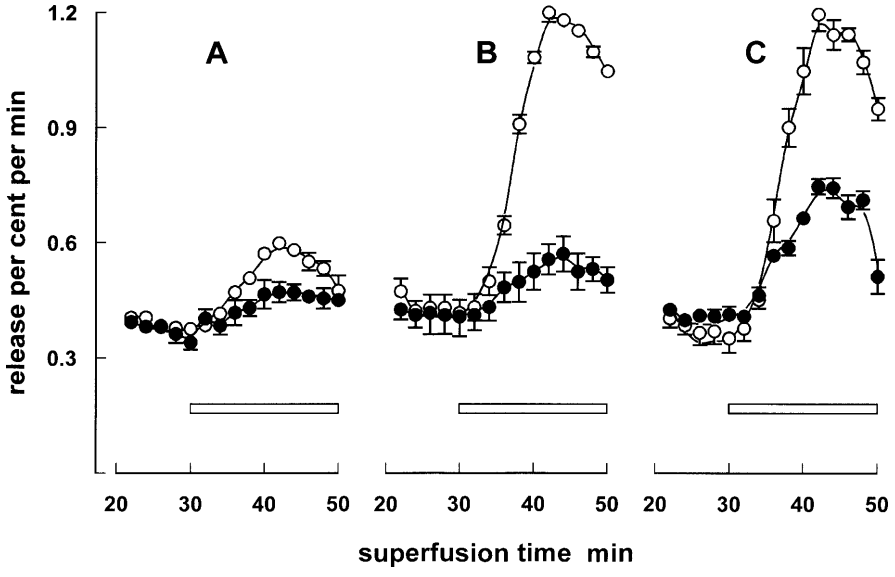

Fig. 3. Effects of TPMPA (0.1 mM) on K^+-stimulated taurine release in hippocampal slices from 3-month-old mice. TPMPA (-●-) was added to the superfusion medium at 30 min as shown by the bar (**A**) together with 50 mM K^+ (-○-), (**B**) with 50 mM K^+ + 0.1 mM CACA (-○-) and (**C**) 50 mM K^+ + 0.1 mM TACA (-○-). Each point is a mean ± SEM of 4 independent experiments. *TPMPA*, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid; *CACA*, cis-4-aminocrotonic acid; *TACA*, trans-4 aminocrotonic acid

Table 2. Effects of $GABA_B$ compounds on taurine release from mouse hippocampal slices

Compound (mM)	Efflux rate constants k_2 (34–50 min) ± SEM (% of k_1)	
	3-month-old	7-day-old
None (control)	82.9 ± 2.9 (14)	84.7 ± 2.3 (13)
Baclofen 0.01	80.5 ± 5.5 (4)	83.4 ± 2.2 (4)
Baclofen 0.1	102.4 ± 5.3** (8)	118.9 ± 11.1** (14)
+phaclofen 0.1	103.3 ± 6.1** (4)	105.0 ± 7.1** (4)
Phaclofen 0.1	94.8 ± 3.4 (4)	88.8 ± 4.7 (4)
Saclofen 0.1	90.1 ± 5.7 (4)	95.8 ± 4.3* (4)
SKF 97541 0.01	92.1 ± 4.8 (4)	110.8 ± 16.4* (4)
SKF 97541 0.1	103.7 ± 3.2** (8)	105.0 ± 5.1** (4)
+saclofen 0.1	106.2 ± 6.5** (4)	112.7 ± 10.9** (4)
3-APPA 0.1	110.3 ± 9.0** (4)	101.5 ± 3.0** (4)

The slices were first 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 the above effectors. The results are percentages of the basal efflux rate constant (k_1) of each slice. Number of independent experiments in parentheses. Significance of differences from the corresponding controls: *$p < 0.05$, **$p < 0.01$. *3-APPA*, 3-aminopropylphosphonic acid; *SKF 97541*, 3-aminopropyl(methyl)phosphinic acid.

GABA or taurine acting on these receptors. Moreover, it could be mediated by a reversal of the direction of Na^+-dependent transporters or by heteroexchange processes. Nor is the possibility of GABA acting on taurine autoreceptors excluded, although so far there is no firm evidence of the existence of such receptors (Saransaari and Oja, 1992). The GABAergic compounds tested on taurine release were chosen to represent the three known types of GABA receptors (Bormann and Feigenspan, 1995; Misgeld et al., 1995; Johnston, 1996a,b). This GABA group appears to be the most complicated family of receptors in terms of the large number of receptor subunits and the variety of ligands with specific sites on receptors. Many of the agents have overlapping actions on different GABA receptors and subunits, and could additionally be inhibitors of GABA uptake. For example, the bicyclic isoxale THIP is a $GABA_A$ agonist with an antagonist potency at $GABA_C$ receptors, but it does not influence GABA uptake. On the other hand, the isothiouronium analogue of GABA, ZAPA, is a substrate for GABA transport, an agonist of low-affinity $GABA_A$ receptors and also a $GABA_C$ agonist. SKF 97541 is a potent $GABA_B$ agonist and also a $GABA_C$ antagonist (see Misgeld et al., 1995; Johnston 1996a,b).

All three types of GABA receptors are known to exist in the hippocampus (Schmid et al., 1996; Francis et al., 1999) and could thus modify taurine

Table 3. Effects of $GABA_C$ compounds on taurine release from mouse hippocampal slices

Compound (mM)	Efflux rate constants k_2 (34–50 min) ± SEM (% of k_1)	
	3-month-old	7-day-old
None (control)	82.9 ± 2.9 (14)	84.7 ± 2.3 (13)
CACA 0.01	90.5 ± 2.1 (4)	84.8 ± 5.8 (4)
CACA 0.1	103.1 ± 4.5** (4)	115.6 ± 5.0** (4)
+THIP 0.1	108.6 ± 7.5** (4)	150.1 ± 21.7** (4)
+TPMPA 0.1	127.3 ± 5.0** (4)	114.7 ± 7.0** (4)
TACA 0.1	100.1 ± 5.3* (4)	189.7 ± 29.7** (4)
+TPMPA 0.1	113.3 ± 5.6** (4)	152.3 ± 17.1** (4)
TPMPA 0.1	107.4 ± 8.4** (4)	86.1 ± 1.9 (4)

The slices were first 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 the above effectors. The results are percentages of the basal efflux rate constant (k_1) of each slice. Number of independent experiments in parentheses. Significance of differences from the corresponding controls: *$p < 0.05$, **$p < 0.01$. *CACA*, cis-4-aminocrotonic acid; *TPMPA*, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid; *THIP*, 4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridin-3-ol; *TACA*, trans-4-aminocrotonic acid.

release. Furthermore, hippocampal glial cells also express GABAergic receptors (Steinhauser et al., 1994). GABAergic interneurons generate both $GABA_A$- and $GABA_B$-mediated inhibition in the hippocampus (Nurse and Lacaille, 1997). Furthermore, the expression of $GABA_C$ receptor and particularly its ρ subunits has recently been demonstrated in this brain region (Wegelius et al., 1998). During development the composition and properties of GABA receptors differ markedly from those expressed in the adult brain. In the fetal and neonatal hippocampus GABA-activated chloride channels lead to marked membrane depolarization through activation of $GABA_A$ receptors, while $GABA_B$ receptors hyperpolarize CA3 pyramidal cells (Cherubini et al., 1991). This kind of dramatic change was not observed now in the GABA-activated taurine release. The differences between adult and developing mice were often only quantitative in nature. During postnatal development the expression of two known $GABA_B$ receptor splice variants is differently regulated (Fritschy et al., 1999). Moreover, the bicuculline- and baclofen-insensitive type of receptor (Strata and Cherubini, 1994) and ρ subunits (Wegelius et al., 1998) are also evident in the hippocampus during the first few weeks after birth. Taurine is able to interact with $GABA_A$ receptors in synaptic membranes (Medina and DeRobertis, 1984; Malminen and Kontro, 1986) and with baclofen binding to $GABA_B$ sites (Kontro and

Table 4. Effects of GABAergic compounds on potassium-stimulated taurine release from mouse hippocampal slices

Compound (mM)	Efflux rate constants k_2 (34–50 min) ± SEM (% of k_1)	
	3-month-old	7-day-old
K^+ (50) (control)	159.0 ± 6.2 (10)	708.7 ± 69.0 (8)
GABA 0.01	136.4 ± 4.6 (4)	740.3 ± 35.0 (4)
GABA 0.1	146.4 ± 8.7 (4)	1028.2 ± 62.9* (4)
TACA 0.01	159.5 ± 13.3 (4)	–
TACA 0.1	258.0 ± 17.6** (4)	765.9 ± 72.0 (4)
ZAPA 0.1	143.7 ± 6.9 (4)	703.4 ± 39.7 (4)
THIP 0.01	196.3 ± 18.6 (4)	981.4 ± 100.6 (4)
THIP 0.1	123.1 ± 6.1** (4)	805.7 ± 37.5 (4)
Bicuculline 0.1	122.8 ± 6.9** (4)	654.5 ± 31.8 (4)
Baclofen 0.01	147.5 ± 14.8 (4)	770.8 ± 65.5 (8)
Baclofen 0.1	125.2 ± 4.0** (7)	722.4 ± 36.0 (13)
+saclofen 0.1	122.1 ± 8.5** (4)	544.0 ± 59.6 (4)
Phaclofen 0.1	121.8 ± 6.8** (8)	750.8 ± 51.6 (8)
Saclofen 0.1	149.1 ± 24.0 (4)	671.6 ± 12.5 (4)
SKF 97541 0.01	125.1 ± 3.5** (4)	–
SKF 97541 0.1	125.0 ± 4.4** (8)	751.7 ± 76.0 (7)
+saclofen 0.1	119.1 ± 6.4** (4)	605.1 ± 41.4 (4)
CACA 0.01	143.1 ± 6.2 (8)	–
CACA 0.1	322.0 ± 30.0** (4)	793.7 ± 60.5 (8)
+TPMPA 0.1	127.3 ± 5.0** (4)	620.1 ± 76.5 (4)
TACA 0.1 + TPMPA 0.1	160.6 ± 1.7 (4)	536.8 ± 56.2 (4)
TPMPA 0.1	123.0 ± 1.4** (4)	742.8 ± 69.1 (4)

The slices were first 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^+ and the above effectors. The results are percentages of the basal efflux rate constant (k_1) of each slice. Number of independent experiments in parentheses. Significance of differences from the corresponding controls: *$p < 0.05$, **$p < 0.01$.
TACA, trans-4-aminocrotonic acid; *THIP*, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; *ZAPA*, (Z)-3-[(aminoiminomethyl)thio]prop-2-enoic acid; *SKF* 97541, 3-aminopropyl(methyl)phosphinic acid; *CACA*, cis-4-aminocrotonic acid; *TPMPA*, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid.

Oja, 1990). The hippocampal fields CA1, CA2 and particularly CA3 are sensitive to inhibition of muscimol binding by taurine at physiological concentrations, indicating that taurine might act on a certain subpopulation of GABA receptors (Bureau and Olsen, 1991).

The concentration-dependent enhancement of basal taurine release by GABA was not affected by the $GABA_A$ receptor antagonists, nor did these compounds have any effects on the actions of the agonists TACA, ZAPA and THIP in the adults, which would imply that $GABA_A$ receptors are not involved in the release. The situation seems more complicated in the immature hippocampus, where the TACA effect was reduced by ZAPA, also a $GABA_C$ antagonist. On the other hand, the enhancement of basal

Table 5. Effects of GABAergic compounds on taurine uptake in mouse cerebral cortical synaptosomal preparations

Compound (0.1 mM)	Uptake ± SEM (% of control)	
	3-month-old	7-day-old
Control	100.0 ± 3.8 (12)	100.0 ± 5.9 (5)
GABA	75.4 ± 3.0** (8)	64.8 ± 1.4** (4)
TACA	75.4 ± 1.7** (8)	73.0 ± 3.3** (4)
ZAPA	72.0 ± 2.7** (8)	74.2 ± 3.6* (4)
THIP	85.0 ± 2.2** (8)	70.2 ± 5.3** (4)
Bicuculline	77.3 ± 3.7** (8)	70.3 ± 2.0** (4)
Baclofen	79.5 ± 1.7** (8)	86.4 ± 4.2 (4)
Phaclofen	75.4 ± 2.8** (8)	75.5 ± 2.3** (4)
Saclofen	73.5 ± 3.2** (8)	78.7 ± 5.0* (4)
SKF 97541	79.0 ± 5.9** (8)	83.7 ± 1.3* (4)
CACA	72.3 ± 2.7** (8)	67.6 ± 3.7** (4)
TPMPA	83.1 ± 3.1** (8)	78.8 ± 4.2* (4)

Synaptosomal preparations were incubated for 10 min with 10 μM [^{3}H]taurine in Krebs-Ringer-Hepes medium (pH 7.4) in the presence of the above compounds. Number of independent experiments in parentheses. Significance of differences from the corresponding controls: *$p < 0.05$, **$p < 0.01$. Abbreviations: see Tables 1–3.

release by bicuculline at both ages and the reduction of K^+-stimulated release in the adults would indicate the involvement of $GABA_A$ receptors in taurine release. In keeping with this, the potentiations of release by TACA and ZAPA were pronounced, particularly in the developing hippocampus.

The potentiation of basal taurine release by GABA being antagonized by $GABA_B$ antagonists suggests that this release could be mediated by presynaptic $GABA_B$ receptors at both ages. However, the enhancement by baclofen and SKF 97541 was not reduced by the antagonists phaclofen and saclofen, indicating the involvement of further mechanisms. Moreover, both $GABA_B$ agonists and antagonists were similarly able to inhibit the K^+-stimulated release in the adults, which indicates receptor functions. It is generally believed that one of the major functions of $GABA_B$ receptors is to modulate neurotransmitter release, baclofen inhibiting release by different mechanisms (see Misgeld et al., 1995). The interpretations are complicated by the existence of $GABA_B$ receptor subtypes having distinct functions and pharmacological properties (Bonanno and Raiteri, 1993; Mott and Lewis, 1994; Misgeld et al., 1995). This receptor heterogeneity is particularly pronounced in the hippocampus (Fritschy et al., 1999). $GABA_C$ receptors seem to be at least partly able to modify basal taurine release in the hippocampus, although the agonist effects were not influenced by the antagonists. The potentiation of K^+-stimulated release was also partially abolished by TPMPA in the adults, confirming the involvement of these newest GABA receptors in taurine release.

The sodium-dependent taurine transport system, with high- and low-affinity components, is known to operate in brain tissue (Oja and Kontro, 1983; Huxtable, 1992), and a carrier common to GABA, taurine and hypotaurine has been postulated (Kontro and Oja, 1983). To date, a taurine transporter with corresponding properties has recently been cloned from the rat brain (Liu et al., 1992; Smith et al., 1992; Borden, 1996). GABA transporters are also moderately inhibited by taurine (Borden, 1996). Thus, the potentiation of basal taurine release by GABA itself and by most of the GABAergic substances could result from a reversal of direction of transporters at cell membranes and by trans-stimulation. Accordingly, GABA perfusion in vivo greatly enhance the extracellular levels of taurine in the rat hippocampus in a dose-dependent manner (Lerma et al., 1985), and CACA and ZAPA can also act as substrates for GABA transport systems in several brain areas (Allan et al., 1991; Chebib and Johnston, 1997). The cloned taurine transporter is moderately inhibited by GABA (Smith et al., 1992; Borden, 1996), as was taurine uptake in synaptosomal preparations from the adult and developing brain in the present experiments. The rather weak inhibition of taurine release by the tested GABAergic agents may nevertheless imply that these substances could be substrates for the transporters and the enhancement of release partly due to transporters. Such an inference is corroborated by the lack of potentiation by GABA in the absence of Na^+. However, the similar degree of inhibition of uptake is not in agreement with the greater potentiation of release observed in the immature hippocampus. Moreover, heteroexchange alone does not explain the inhibition by the $GABA_B$ and $GABA_C$ receptor antagonists.

In conclusion, taurine release in both adult and developing hippocampus appears to be modulated by GABAergic agents. The potentiation of basal taurine release by GABA was regulated by compounds acting on both $GABA_B$ and $GABA_C$ receptors at both ages, although the involvement of $GABA_A$ receptors could not be excluded. The inhibition of K^+-stimulated release in the adults could be mediated by $GABA_B$ receptors and the enhancement by $GABA_C$ receptors. Furthermore, GABAergic compounds also potentially influence basal taurine release by a reversal of the direction of transporters at both ages.

Acknowledgements

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

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Authors' address: Professor Pirjo Saransaari, Tampere Brain Research Center, University of Tampere Medical School, Box 607, FIN-33101 Tampere, Finland, Fax: +358 3 215 6170, e-mail: blpisa@uta.fi

Received June 28, 1999