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Effects of taurine on GABA release from synaptosomes of rat olfactory bulb

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Summary. Superfusion of synaptosomes prepared from rat olfactory bulb revealed constant basal release of endogenous taurine (Tau), aspartate (Asp), glutamate (Glu) and γ-aminobutyrate (GABA): their release rates were 110.4 ± 13.0, 30.3 ± 6.7, 93.7 ± 13.1, and 53.3 ± 8.8 pmol/min/mg protein, respectively. The depolarizing-stimulation with 30mM KCl evoked 1.17-, 2.18-, 2.55- and 1.53-fold increases, respectively. Tau release was calcium-independent. However, the perfusion of synaptosomes with Tau (10μM) inhibited the evoked increase in GABA release by 63% without changing basal release, although it did not affect release of Asp and Glu. Phaclofen (10μM, a $GABA_B$ receptor antagonist), but not bicuculline (10μM, a $GABA_A$ receptor antagonist), counteracted the Tau-induced reduction in GABA release. These data suggest that Tau may be abundantly released from nerve endings of rat olfactory bulb and that it may regulate GABA release through the activation of presynaptic $GABA_B$ autoreceptors.

Keywords: Amino acids – Taurine – γ-Aminobutyric acid – Synaptosome – Olfactory bulb – Rat

Introduction

Taurine (Tau; 2-aminoethanesulfonic acid) is one of the most abundant amino acids in brain. The high levels of Tau in rat olfactory bulb, cerebellum, and cerebral cortex suggest that the substance may play a specific role as a neurotransmitter or modulator in these regions (Lombardini, 1976). Although Tau has been reported to be released from glial cells by the stimulation of β-adrenoceptors (Martin and Shain, 1993), there still remains a possibility that Tau may be released from neuronal elements because depolarizing-stimulations evoked Tau release in a tetrodotoxin-sensitive fashion (Singewald et al., 1993).

On the other hand, application of Tau produces γ-aminobutyrate (GABA)-like inhibitory currents in neurons of brain slices (Okamoto et al.,

1983; Hausser et al., 1992). Moreover, Tau has been found to interact with both $GABA_A$ and $GABA_B$ receptor binding sites (Medina and De Robertis, 1984; Malminen and Kontro, 1986; Kontro and Oja, 1990; Bureau and Olsen, 1991). The membrane-stabilizing effects of Tau, such as its anticonvulsant activity, appear to be mediated through GABA receptors. However, several lines of evidence demonstrated that Tau possesses many distinct actions from those of GABA, such as development and survival of neuronal cells (Hayes et al., 1975), modulation of calcium fluxes and transmitter release (Namima et al., 1983; Huxtable, 1989), and regulation of osmolarity (Wade et al., 1988) in the mammalian brain.

Perfusion-experiments of synaptosomes suggested the existence of presynaptic heteroreceptors which regulate release of distinct neurotransmitters, in addition to autoreceptors. For example, α_2 adrenoceptors are present on nerve terminals of excitatory amino acid neurons in various regions where they inhibit the depolarization-evoked release of aspartate (Asp) and glutamate (Glu) (Kamisaki et al., 1991; 1992). Therefore, the present studies using synaptosomes prepared from rat olfactory bulb were designed to clarify (1) whether Tau can be released by depolarizing-stimulation from nerve terminals; (2) whether Tau can regulate the basal and depolarization-evoked release of Asp, Glu and GABA from synaptosomes; and (3) if so, which types of presynaptic receptors may be involved.

A part of this manuscript has been published in an abstract form at the 4th International Congress on Amino Acids (Kamisaki et al., 1995).

Materials and methods

Preparation of synaptosomes

Male Wistar rats (170–200 g; SLC Inc., Shizuoka) were sacrificed according to guidelines specified by the animal use committee of Tottori University. Both axosomatic/dendritic and dendrodendritic synaptosomes were prepared according to Rochel et al. (1981) with minor modifications (Kamisaki et al., 1991; 1993). Olfactory bulbs were separated and homogenized in 20 volumes of 0.32 M sucrose buffered with 20 mM Tris-HCl (pH 7.5) with a Dounce homogenizer. After centrifugation at 1,000 g for 5 min, the upper buffy zone of the pellet was combined with the supernatant and centrifuged at 12,000 g for 20 min. The resulting pellet was suspended in a Krebs-Ringer buffer (KRB, composition in mM: NaCl, 135; KCl, 4.8; $CaCl_2$, 2; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; HEPES, 12.5; glucose, 10; pH 7.4) previously equilibrated with O_2/CO_2 (95:5). The prepared homogenates and synaptosomes were mixed with trichloroacetic acid (final concentration of 5%, v/v), centrifuged and used for the determination of amino acid content. The protein content was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Release of amino acids

Synaptosomes (ca. 0.8 mg protein) were superfused as described by Kamisaki et al. (1994). They were layered on a filter with a gel (Sephadex G-10) and perfused with KRB aerated continuously with O_2/CO_2 (95:5) at a flow rate of 0.5 ml/min at 37°C. The superfusate was subsequently collected at 5 min intervals into a tube containing

1.5 nmol of 5-aminopentanoic acid as an internal standard and kept frozen at −80°C until assayed. Forty min after the start of superfusion the synaptosomes were stimulated by 30 mM KCl for 2 min, replacing an equimolar concentration of NaCl. Tau and various agents (10 μM) were added to KRB 10 min before the stimulation. In calcium-free experiments, the medium was replaced by KRB containing 1.0 mM ethylene glycol bis(β-aminoethylether)-N,N′-tetraacetic acid instead of 4.8 mM $CaCl_2$ after 30 min of perfusion.

Determination of amino acids

The content of Asp, Glu, Tau, and GABA in preparations and perfusate was measured by HPLC using a gradient elution system (Kamisaki et al., 1990; 1993). The analytical method involved precolumn derivatization with o-phthaldialdehyde and 2-mercaptoethanol followed by separation on a reversed-phase column with two mobile phases of 40 mM NaH_2PO_4 buffer (pH 7.00)-acetonitrile-methanol (90:9:1, v/v) and of 100 mM Na citrate buffer (pH 3.50)-acetonitrile-methanol (59:32:9, v/v) at a flow rate of 1.5 ml/min. The concentrations were determined from relative fluorescence intensities.

Calculation and statistical analysis

The content of amino acids in the fractions of perfusate is expressed as a release rates of pmol/min/mg protein of synaptosomes (means ± S.D.). The basal and evoked release rates of amino acids were the average amount in two 5-min fractions before KCl stimulation and that in the 5-min fraction during depolarization, respectively. For the statistical analysis, after one-way or multiway ANOVAs, Student's t or Dunnett's test was adopted to compare the source of data obtained from two or multiple groups. Differences were considered significant when $P < 0.05$.

Results

Amino acid composition in rat olfactory bulb

The content of amino acids in rat olfactory bulb is listed in Table 1 and compared with those in rat cerebral cortex. Tau content was highest in the

Table 1. The content of amino acids in homogenates and synaptosomes prepared from rat olfactory bulb

		Olfactory bulb	Cerebral cortex
Whole homogenates	Taurine	83.3 ± 6.3	31.7 ± 3.2
	Aspartate	16.7 ± 2.1	8.3 ± 1.3
	Glutamate	76.2 ± 9.6	87.1 ± 3.3
	γ-Aminobutyrate	31.8 ± 2.4	8.3 ± 0.9
Synaptosomes	Taurine	32.3 ± 4.3	25.2 ± 2.1
	Aspartate	10.5 ± 1.6	7.1 ± 1.2
	Glutamate	38.8 ± 6.8	66.2 ± 9.8
	γ-Aminobutyrate	14.6 ± 2.7	8.2 ± 1.0

Extracts in trichloroacetic acid were analyzed by HPLC. The content of amino acids is expressed as nmol per mg protein (means ± S.D.) from 5 experiments.

olfactory bulb, although Glu content was highest in the cerebral cortex. Synaptosomal fractions possess Tau, the second most abundant substance after Glu in both regions, suggesting the possibility that Tau may be released by depolarizing-stimulation and function as a neurotransmitter similar to Glu. In the olfactory bulb, however, the comparison between Tau content in the whole tissue and synaptosomes indicates that Tau is not concentrated in the typical fractions of nerve endings.

Release of Tau from superfused synaptosomes and its calcium dependency

Figure 1 illustrates release of Tau from synaptosomes of rat olfactory bulb before, during and after the depolarization. Superfusion with KRB revealed a

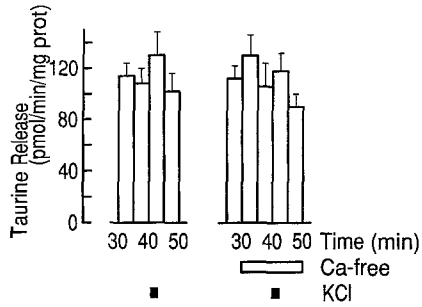

Fig. 1. The basal and evoked release of Tau from synaptosomes of rat olfactory bulb. The crude synaptosomal fraction was continuously superfused with KRB. Forty min after the start of perfusion, synaptosomes were exposed to 30 mM KCl for 2 min (indicated by solid bars). Tau released into the perfusate was determined by HPLC and presented as a rate of pmol per min per mg protein of synaptosomes (left series of columns). When calcium ions were omitted from KRB 10 min before the depolarization (indicated by open bars), release rates of Tau are expressed on the right. Values are means ± S.D. from 4–10 separate experiments

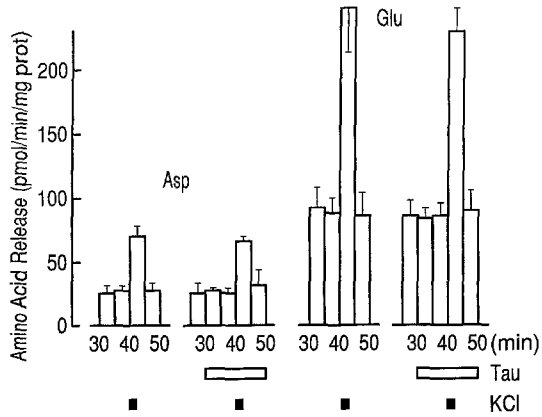

Fig. 2. Effects of Tau on release of excitatory amino acids from synaptosomes. The preparations were superfused with KRB and depolarized by high potassium medium (indicated by solid bars). Tau (10 μM; indicated by open bars) was added to KRB 10 min before the stimulation. Release rates of aspartate (*Asp*) and glutamate (*Glu*) are expressed as means ± S.D. from 7–8 separate experiments. Note, no significant differences exist between release rates in the absence and presence of Tau

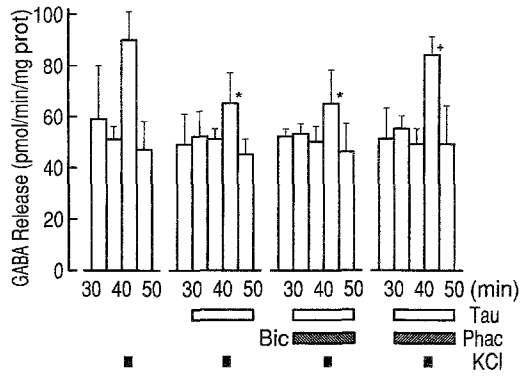

Fig. 3. Effects of Tau on release of γ-aminobutyrate (*GABA*) from synaptosomes. Olfactory bulb synaptosomes were superfused and stimulated as described in the legend of Fig. 1. Tau (10μM; indicated by open bars) and GABA receptor antagonists (indicated by shaded bars), bicuculline (*Bic*; 10μM) or phaclofen (*Phac*; 10μM), were added to KRB 10min before the depolarization. Each value is the mean ± S.D. from 4–8 experiments. *$P < 0.01$, compared with that in the absence of Tau. $^{+}P < 0.05$, compared with that in the presence of Tau alone

higher basal release rate of Tau (110.4 ± 13.0pmol/min/mg protein of synaptosomes; n = 10), compared with that of Asp, Glu and GABA (Figs. 2 and 3). A 2-min stimulation with 30mM KCl failed to evoke a significant increase in Tau release. Moreover, the removal of calcium ions from KRB did not affect either the basal nor evoked release of Tau. Therefore, these data indicate that a large amount of Tau may be constantly released from nerve terminal preparations but not by high potassium stimulation.

Effects of Tau on release of Asp, Glu and GABA

The basal release rates of Asp, Glu and GABA were 30.3 ± 6.7, 93.7 ± 13.1 and 53.3 ± 8.8pmol/min/mg protein, but were evoked by KCl stimulation to levels of 66.0 ± 10.0, 239.1 ± 33.6 and 81.7 ± 8.9pmol/min/mg protein, respectively (n = 8) (Figs. 2 and 3). Although the release rates of Asp and Glu from olfactory bulb synaptosomes were almost consistent with results in cerebral cortex, that of GABA was approximately 3-fold of that in the cortex (Kamisaki et al., 1993). When calcium ions were omitted from the perfusion medium 10min before depolarization, basal rates of release did not change (data not shown). However, depolarization-evoked rates were reduced: Asp, 47.6 ± 4.4; Glu, 132.4 ± 22.1; GABA, 73.9 ± 8.8pmol/min/mg protein, indicating that the calcium dependent portions of the evoked increases were 52, 73 and 27%, respectively. The reductions in the evoked release of Asp and Glu were significant ($P < 0.01$).

The addition of Tau (10μM) to KRB did not affect either the basal release of Asp and Glu (27.3 ± 3.0 and 85.9 ± 10.6pmol/min/mg protein) or the evoked release (67.2 ± 4.1 and 229.1 ± 16.8pmol/min/mg protein, respectively; n = 7) (Fig. 2). However, Tau significantly reduced the evoked release of GABA to 65.4 ± 13.4pmol/min/mg protein (n = 7), although

the basal release of GABA was not affected (51.9 ± 7.0 pmol/min/mg protein) (Fig. 3).

Effects of GABA receptor antagonists

The inhibitory effect of Tau (10μM) on depolarization-evoked release of GABA was significantly inhibited by phaclofen (10μM, a $GABA_B$ receptor antagonist) (84.3 ± 7.2 pmol/min/mg protein; n = 4) (Fig. 3). However, bicuculline (10μM, a $GABA_A$ receptor antagonist) did not attenuate the effects of Tau on the evoked release of GABA (64.8 ± 13.7 pmol/min/mg protein; n = 4). Neither antagonist modified basal release in the presence of Tau. Moreover, these antagonists did not show any significant effect on basal and evoked release of amino acids in the absence of Tau (data not shown).

Discussion

In the present experiments, crude synaptosomal fractions were prepared from rat olfactory bulb. The preparations were reported to contain two types of synaptosomes: axosomatic/dendritic and dendrodendritic nerve endings (Quinn and Cagan, 1980; Rochel et al., 1981). Since Tau has been reported to be released from glial cells by receptor mediated stimulation (Martin and Shain, 1993), it is rather difficult to discuss the subcellular localization of Tau without further fractionation of the crude synaptosomes. However, the concentration of Tau in the synaptosomal fraction was higher than all other amino acids except Glu (Table 1). These data suggest that Tau may be stored not only in glial and neuronal cell bodies, but also at nerve terminals in the rat olfactory bulb. In addition, there is the possibility that Tau, like Glu, may play an important role as a neurotransmitter.

The released content of Tau from the synaptosomes into perfusate was the most abundant among neurotransmitter amino acids under unstimulated conditions (Figs. 1–3). However, high potassium stimulation evoked no significant increase in Tau release from synaptosomes of olfactory bulb. To date, it has not been established whether depolarization may elicit Tau release from brain tissue. An in vivo dialysis experiment revealed that depolarization with either high potassium or veratridine evoked a 2.5–3.5 fold increase in Tau release in the hypothalamus and that Tau release was reduced by treatment with tetrodotoxin (Singewald et al., 1993). On the other hand, stimulation with KCl caused no effect on Tau efflux from cerebral cortex slices (Low, 1994). In our previous experiments, KCl stimulation evoked only a 1.3-fold increase in Tau release from synaptosomes of rat cerebral cortex (unpublished observation). The present data in the olfactory bulb are consistent with the latter, suggesting that Tau may not be released from nerve terminals by a depolarizing nerve impulse, a mechanism expected for a neurotransmitter.

Nonetheless, it has been reported that Glu receptor agonists elicit an increase in extracellular Tau levels through the activation of N-methyl-D-aspartic acid receptors (Menéndez et al., 1989; Shibanoki et al., 1993). Therefore, Tau may play an important role as a neuromodulator whose release is probably regulated through the activation of presynaptic receptors located in Tau-containing nerve terminals.

Because of the high levels of Tau in olfactory bulb, we investigated whether Tau may regulate release of other amino acid transmitters from nerve terminals. The synaptosomal preparations were superfused with KRB in the absence or presence of Tau. Tau failed to affect basal release of Asp, Glu and GABA. However, the depolarization-evoked increase in GABA release was significantly attenuated by Tau, although those in Asp and Glu release were not (Figs. 2 and 3). These results are different from those obtained in rat cerebral cortex, where Tau inhibited the evoked release of both Asp and Glu, in addition to GABA (Kamisaki et al., 1993). The reason for this discrepancy is unclear, but it may be due to differences in the preparation of synaptosomes containing dendrodendritic terminals in the olfactory bulb. However, the inhibitory effects of Tau on GABA release is in accordance with previous reports that Tau inhibits GABA release from brain tissue (Namima et al., 1983; Huxtable, 1989; Kamisaki et al., 1993). Since Tau has been reported to interact with GABA receptors which are classified as $GABA_A$ and $GABA_B$ subtypes (Medina and De Robertis, 1984; Malminen and Kontro, 1986; Kontro and Oja, 1990; Bureau and Olsen, 1991), we discriminated the effect of Tau by addition of the specific GABA receptor antagonist. The Tau-induced reduction in GABA release was blocked by a $GABA_B$ antagonist, phaclofen, but not by a $GABA_A$ antagonists, bicuculline (Fig. 3), an observation consistent with the result in cerebral cortex. Therefore, the present experiments using synaptosomal fractions suggest that Tau-induced inhibition is mediated through $GABA_B$ autoreceptors located at presynaptic sites of GABAergic-nerve terminals.

It is possible that endogenously released Tau may interfere with the release of GABA under control conditions. However, this is unlikely in our system which separates synaptosomes with gel and continuously washes them with KRB to prevent interference of released substances with synaptosomes (Kamisaki et al., 1994). When the synaptosomes were superfused in the presence and absence of phaclofen alone, there was no significant difference between the amount of GABA released. In addition, our previous experiments using cortical synaptosomes also revealed no interaction of released substances, since selective antagonists alone showed no effect on amino acid release (Kamisaki et al., 1991; 1933). Therefore, exogenously applied Tau is able to act upon the synaptosomal preparations to inhibit the evoked release of GABA.

In conclusion, the present observations provide evidence that Tau may play an important role as a neuromodulator in regulating the depolarization-evoked release of GABA through the activation of presynaptic $GABA_B$ receptors in rat olfactory bulb.

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