A MODULATING ROLE OF TAURINE ON RELEASE OF ACETYLCHOLINE AND NOREPINEPHRINE FROM NEURONAL TISSUES

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Abstract—Effects of taurine (2-aminoethanesulfonic acid) on the uptake and release of \(^{14}\text{C}\)-acetylcholine (\(^{14}\text{C}\)-ACh) and \(^{3}\text{H}\)-norepinephrine (\(^{3}\text{H}\)-NE) in the superior cervical ganglion and cerebral cortex of the rat were studied. Taurine suppressed high potassium evoked release of \(^{14}\text{C}\)-ACh and \(^{3}\text{H}\)-NE from the rat superior cervical ganglia and cerebral cortical slices, while the drug did not modify per se the uptake and unstimulated (spontaneous) release of \(^{14}\text{C}\)-ACh and \(^{3}\text{H}\)-NE in these tissues. Furthermore, taurine inhibited the release of \(^{3}\text{H}\)-NE from the crude synaptosomal (P2) fraction of the rat brain without affecting the uptake. These results suggest that taurine may act as a modulator of neuronal activity, possibly by stabilizing excitable membrane and by suppressing the release of neurotransmitter at synapses.

Taurine is present in the mammalian central nervous system (CNS) in relatively large quantities, but the function has not been clearly defined. Although many reports (1-4) are available describing the neuropharmacology of taurine, there are discrepancies concerning its mechanism of action in the brain. Evidence such as uneven distribution of taurine in the CNS (5), synaptosomal enrichment of taurine and cysteine sulfinate decarboxylase activity (6), synaptosomal high affinity uptake of taurine (7), electrical stimulation (2, 8) or high potassium (8)-induced release of taurine from the cerebral cortical slices, and depressant action of taurine on various CNS neurons (9-12) support the idea that this compound may act as a specific neurotransmitter in mammalian CNS. On the other hand, evidence suggesting a modulator role of taurine for membrane excitability such as glial uptake (13), postnatal decline in cerebral content of taurine (14), increased mitochondrial association of calcium in the presence of taurine (15, 16), increased potassium transport by taurine (16), lack of electrically stimulated release of taurine from the rat cerebral cortical slices (17), and lack of effects of taurine on cerebral and cerebellar cortical synapses (18, 19) have also been reported. These facts indicate that studies aimed at accumulating more evidence that taurine actually acts on central synapses as a neurotransmitter or neuromodulator are essential to clarify the mechanism of action of taurine in the brain.

The present study was undertaken to determine whether or not taurine may act as a modulator of neuronal activity, possibly by altering the release of neurotransmitter candidates from excitable tissues.
MATERIALS AND METHODS

Male Wistar rats weighing 150-200 g were used throughout and were deprived of all food for 12 hr before each experiment. Crude synaptosomal (P2) fraction from the rat brain was prepared according to the method of Gray and Whittaker (20). DL-[7-3H] norepinephrine (3H-NE; S.A.: 12 Ci/mmol) and Acetyl [N-methyl-14C]choline chloride (14C-ACh; S.A.: 20.4 mCi/mmol) were obtained from the Radiochemical Centre, Amersham.

Uptake and release of 3H-NE: Cortical slices (coronal section, thickness: 0.35-0.4 mm), crude synaptosomal (P2) fractions from the cerebral cortex and chopped superior cervical ganglia were used. These tissues were weighed, and packed respectively with a nylon mesh. The package of cerebral cortical slices (10-30 mg wet weight) or chopped superior cervical ganglia (1.7-2.6 mg wet weight) was initially incubated at 37°C in 1.0 ml of oxygenated Krebs-Ringer Tris-HCl (pH 7.4) containing 10 μM pargyline. Following the preincubation for 10 min, 5 μl of aqueous solution containing 0.1-0.2 nmoles of 3H-NE were added and further incubated for 30 min (cerebral cortical slices) or 60 min (ganglia) to preload the tissues with 3H-NE. After being washed twice with Krebs-Ringer Tris-HCl (pH 7.4) containing 10 μM pargyline, each tissue was transferred successively to vials containing 2.0 ml of Krebs-Ringer Tris-HCl (pH 7.4) and 10 μM pargyline, and the radioactivity of each vial was counted to determine the release of 3H-NE. The rate of 3H-NE release was calculated by the following equation:

\[
\frac{\text{d.p.m. released into medium per 2 min}}{\text{total d.p.m. released into medium} - \text{d.p.m. remaining in tissue}} \times 100
\]

At the end of release measurements, radioactivity remaining in the tissues was determined after solubilization with Hyamine-methanol. The rate of 3H-NE uptake was calculated from the sum of the remaining radioactivity in the tissue and total radioactivities released into the medium. In experiments to determine the effect of high concentrations of KCl, the same concentrations of NaCl were subtracted from the medium. For determination of the synaptosomal (P2) release of norepinephrine, crude synaptosomal (P2) fractions preloaded with 3H-NE were used. P2 fraction from the brain (6-8 mg protein) was initially incubated with 2.5 ml of Krebs-Ringer Tris-HCl (pH 7.4) containing 10 μM of pargyline and 40 nM of 3H-NE at 37°C for 10 min. After the incubation, 3.0 ml of ice-cold Krebs-Ringer Tris-HCl (pH 7.4) solution was added to each centrifuge tube used for the incubation and each sample was immediately centrifuged at 17,000 x g for 10 min (4°C). After the pellet was washed once by resuspending with 6.0 ml of 0.32 M sucrose and recentrifuging as above, the resultant pellet was finally suspended with 2.0 ml of 0.32 M sucrose. A part of the suspended pellet was solubilized with Hyamine-methanol and subjected to the measurement of radioactivity (determination of 3H-NE uptake) and the remaining suspension was further used for determinations of the release of 3H-NE. For assays of the rate of release, 0.1 ml of suspension was added to 1.9 ml of Krebs-Ringer Tris-HCl (pH 7.4) (final protein concentration: 300-400 μg/ml) and incubated at 37°C for various time intervals. After completion of the incubation, each sample was recentrifuged and radioactivities in the
supernatant and pellet were determined using the same procedures as described in experiments for determining \(^3\)H-NE uptake. The radioactivity was measured with 10 ml of Bray's scintillator (21) using a Packard Trib-Carb liquid scintillation spectrometer, Model 3390. In each release or uptake experiment for \(^3\)H-NE, it was confirmed paper chromatographically (35) that more than 95\% of the radioactivity remains as \(^3\)H-NE. In experiments examining the effects of taurine, leucine and methionine, these amino acids were added to the incubation medium throughout the experiments of uptake and release.

**Uptake and release of \(^14\)C-ACh:** For the measurement of uptake and release of \(^14\)C-ACh in cerebral cortical slices and superior cervical ganglia, essentially the same procedures as described in the case of \(^3\)H-NE were used. In these cases, 50 nmoles of \(^14\)C-ACh was added instead of \(^3\)H-NE, and oxygenated Krebs-Ringer Tris-HCl (pH 7.4) containing 50 \(\mu\)M DFP was used as the reaction mixture. Under these experimental conditions, more than 95\% and 85\% of accumulated and released radioactivities respectively were identified with thin layer chromatographically (34) as being \(^14\)C-ACh.

**Assays of protein and taurine:** Protein contents were determined by the method of Lowry et al (22). Taurine contents were assayed spectrofluorometrically as previously described (23) after extraction by 0.1 N HCl. Each tissue examined was homogenized and centrifuged at 10,000 \(\times\) g for 30 min, and the resultant pellet was washed once by repeating the same procedure. Supernatants obtained by the initial centrifugation and washing procedure were combined and subjected to the assay for taurine.

**Data analysis:** Statistical analyses of results were made by application of Student's t-test. Changes were considered significant when \(P\) was 0.05 or less.

**RESULTS**

**Effects of taurine on uptake and release of \(^14\)C-ACh**

Kinetic order of the release is still undefined. In spontaneous release experiments, the released percent of each fraction on a logarithmic scale against time after beginning of the release yielded curves which could be fitted by the sum of major double exponential terms. The curve consisted of rapid and slow components.

**Fig. 1.** Effect of taurine on KCl (60 mM)-induced increase in release of \(^14\)C-acetylcholine (\(^14\)C-ACh) from rat cerebral cortical slices and superior cervical ganglion. Averages obtained are from 4-5 separate experiments.
Rat superior cervical ganglia (SCG) preloaded with $^{14}$C-ACh showed a time dependent decline of the release in the slow component having the fractional rate constant ($k$) of $0.0379 \text{ min}^{-1}$ and the time required for declining to the half value of the component ($t_{1/2}$) of 18.30 min (Fig. 1). These results are similar to the pattern of $^3$H-GABA (24) or $^3$H-NE (25) release found in brain slices. Addition of 60 mM KCl evoked a significant increase of $^{14}$C-ACh release (482%) and clarified the time required for decline to the half value of maximum release of $^{14}$C-ACh evoked by the addition of KCl ($T_{1/2} \max$) of 10.10 min (Table 1). In vitro addition of 30 mM of taurine or oral administration of taurine for 3 days significantly attenuated the high-potassium evoked $^{14}$C-ACh release from the SCG without affecting the unstimulated (spontaneous) release of $^{14}$C-ACh (Fig. 1). The high-potassium stimulated $^{14}$C-ACh release from the SCG was significantly suppressed or abolished by the removal of calcium from the medium, EGTA treatment, the addition of 20 mM MgCl$_2$, or the addition of 1 mM verapamil, a calcium antagonist (Table 2). These results suggest that the KCl stimulated release of $^{14}$C-ACh from the SCG was calcium dependent and taurine probably inhibited this calcium dependent release of $^{14}$C-ACh. As shown in Table 1, suppressive effects of taurine on the KCl-evoked release of $^{14}$C-ACh from the SCG were not reproduced by equimolar concentrations of leucine or methionine. These data also suggest that this suppressive effect on the KCl evoked release of $^{14}$C-ACh may be specific for taurine. Similarly in vitro addition of taurine (30 mM) significantly inhibited the KCl

### Table 1. Effects of taurine, leucine and methionine on K$^+$-stimulated release of $^{14}$C-ACh and $^3$H-NE

| Superior Cervical Ganglion | % increase by KCl (60 mM) | S.E. | $T_{1/2 \ max}$*# (min) | S.E. |
|-----------------------------|--------------------------|-----|-------------------------|-----|
| Control                     | 482 ± 25                 |     | 10.10 ± 1.28            |     |
| Taurine treated*            | 360 ± 22*                |     | 10.53 ± 0.07            |     |
| Taurine added (10 mM)       | 333 ± 84                 |     | 10.53 ± 0.18            |     |
| Taurine added (30 mM)       | 221 ± 22**               |     | 11.40 ± 0.20            |     |
| Leucine added (30 mM)       | 449 ± 74                 |     | 10.10 ± 0.54            |     |
| Methionine added (30 mM)    | 395 ± 57                 |     | 9.15 ± 1.09             |     |

| Cerebral Cortical Slices    | % increase by KCl (60 mM) | S.E. | $T_{1/2 \ max}$*# (min) | S.E. |
|-----------------------------|--------------------------|-----|-------------------------|-----|
| Control                     | 90 ± 2                   |     | 4.53 ± 0.05             |     |
| Taurine added (30 mM)       | 58 ± 2*                  |     | 4.10 ± 0.17             |     |

| $^3$H-NE                    | % increase by KCl (24 mM) | S.E. | $T_{1/2 \ max}$*# (min) | S.E. |
|-----------------------------|--------------------------|-----|-------------------------|-----|
| Control                     | 457 ± 20                 |     | 2.92 ± 0.14             |     |
| Taurine added (30 mM)       | 351 ± 15*                |     | 3.50 ± 0.18             |     |
| Leucine added (30 mM)       | 443 ± 18                 |     | 3.50 ± 0.17             |     |

Mean ± S.E. obtained from 3–6 separate experiments are shown. *: Taurine was given orally, 4–7 g/kg/day for 3 days. Taurine contents in the brain and superior cervical ganglion (SCG) of these animals were as follows: Control brain; 5.65 ± 0.70, Brain from taurine-treated animals; 6.95 ± 1.77, Control SCG; 3.91 ± 1.22, and SCG from taurine-treated animals; 5.62 ± 0.25 μmol/g w.w., S.E., respectively (N = 4). #: Time required for decline to half value of the maximum release of each neurotransmitter evoked by addition of KCl. *p < 0.05, **p < 0.02.
(60 mM)-evoked release of $^{14}$C-ACh from the $^{14}$C-ACh preloaded cerebral cortical slices without affecting that from unstimulated slices (Fig. 1, Table 1). On the other hand, taurine added in vitro as well as being administered orally for 3 days to the rat had no significant effect on the uptake of $^{14}$C-ACh by SCG or cerebral cortical slices (Table 3).

**Effect of taurine on uptake and release of $^{3}$H-NE**

Rat cerebral cortical slices preloaded with $^{3}$H-NE also showed a time dependent decline
of the release in the slow component having the fractional rate constant \( k \) of 0.0273 min\(^{-1}\) and the time required for declining to the half value of the component \( t_{1/2} \) of 25.48 min (Fig. 2). Addition of high concentration of KCl (24 mM) evoked a significant increase of \(^3\)H-NE release (457%) and clarified the time required for declining to the half value of maximum release of \(^3\)H-NE evoked by the addition of KCl \( T_{1/2 \text{ max}} \) of 2.92 min (Table 1).

In the case of high-potassium stimulated \(^3\)H-NE release from the cerebral cortex, the release was also significantly suppressed or abolished by the removal of calcium from the medium, EGTA treatment, the addition of 20 mM MgCl\(_2\) or the addition of verapamil (0.01–1.0 mM) (Table 2). In vitro addition of 30 mM of taurine significantly inhibited the high potassium evoked release of \(^3\)H-NE from the cerebral cortical slices without affecting the unstimulated release.
(spontaneous) release of \(^{3}\)H-NE (Fig. 2). This inhibitory effect of taurine on high potassium evoked release of \(^{3}\)H-NE also could not be duplicated by the isomolar concentration of leucine (Table 1). Fig. 3 shows the suppressive effects of taurine (30 mM) on KCl-stimulated release of \(^{3}\)H-NE from the cerebral cortical slices as a function of KCl concentration in the medium. Taurine showed statistically significant inhibitions on \(^{3}\)H-NE release when 24 or 60 mM of KCl was added, whereas this suppressive effect of taurine on the KCl-evoked \(^{3}\)H-NE release was not detected in the presence of 12 or 125 mM KCl. These results suggest that occurrence of the suppressive effect of taurine on the release of \(^{3}\)H-NE depends on the state of membraneous excitability and may be counteracted easily by the increase and/or decrease of excitability of neuronal membrane. The effect of taurine on the release of \(^{3}\)H-NE from crude synaptosomal (P₂) fraction of the brain is presented in Fig. 4. In vitro addition of taurine resulted in a statistically significant inhibition of the release of \(^{3}\)H-NE from P₂ as determined 6–20 min after the initiation of incubation. In contrast with significant suppressive effects of taurine on the release of \(^{3}\)H-NE, the uptake of \(^{3}\)H-NE by cerebral cortical slices, crude synaptosomal (P₂) fractions from the brain and superior cervical ganglia was not significantly affected by in vitro addition of taurine (30 mM) or administration of taurine in vivo for 3 days (Table 3).

### DISCUSSION

Taurine apparently has several types of action on excitable membrane. The major interest in the actions of taurine in the mammalian CNS, however, is focused on whether or not this compound actually plays the role of neurotransmitter. In fact many experimental
findings seem to fulfill the criteria for identifying a transmitter: uneven distribution (5), enrichment in nerve terminals (6), synaptosomal high affinity uptake for the removal following the release (7), stimulation evoked release (8, 17, 26), and depressant actions on various CNS neurons (9–12).

Another concept is that taurine may be a modulator of membrane excitability. Investigators such as Huxtable and Bressler (27), van Gelder (28), and Barbeau and Donaldson (29) seem to favor the view that taurine acts as a modulator of membrane excitability. In addition Kuriyama and Nakagawa (4) have shown that administrations of taurine both in vivo and in vitro inhibit the release of epinephrine from adrenal chromaffin granules.

One of the important findings in our study herein is that taurine has significant suppressive effects on the high potassium evoked release of other neurotransmitter candidates such as ACh and NE from a variety of neuronal tissues. Of particular interest is that taurine also acts in such a way in the superior cervical ganglia, where ACh is well established as a neurotransmitting substance. In autonomic ganglia, it has been reported that newly synthesized ACh is released before equilibration with preformed stores (30). Since we utilized superior cervical ganglion prelabeled with 14C-ACh, the effect of taurine on the release of newly synthesized ACh within the ganglia remains to be elucidated. On the other hand, taurine did not modify the uptake and unstimulated (spontaneous) release of ACh and NE in these tissues per se. These results strongly suggest that in both the central and peripheral nervous systems taurine probably acts as a modulator of neuronal activity, possibly by stabilizing excitable membranes and by suppressing the release of neurotransmitter at synapses. Molecular mechanisms underlying these suppressive effects of taurine on the release of neurotransmitter candidates are not clear at present. Our findings herein, however, clearly indicate that the suppressive effect of taurine appears only during the stimulated release of neurotransmitters which in itself requires the presence of calcium. It is well known that calcium plays important roles in regulating the excitability of neuronal tissues by changing the release of neurotransmitter (31, 32) and/or permeability to sodium (33) and potassium. On the other hand, it has been reported that taurine increases mitochondrial association of calcium in the heart and liver (15, 16), and recently acquired data also indicate that a similar phenomenon occurs in brain mitochondria (Kuriyama et al, unpublished). Thus taurine apparently induces suppression of the release of calcium from brain mitochondria and subsequent declines of intracellular free calcium may also contribute at least in part to the suppressive effects of this compound on the release of neurotransmitters at synapses.

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