Spontaneous and Evoked Release of [3H]Taurine from a P2 Subcellular Fraction of the Rat Retina

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The effects of spontaneous and evoked [3H]taurine release from a P2 fraction prepared from rat retinas were studied. The P2 fraction was preloaded with [3H]taurine under conditions of high-affinity uptake and then examined for [3H]taurine efflux utilizing superfusion techniques. Exposure of the P2 fraction to high K+ (56 mM) evoked a Ca2+-independent release of [3H]taurine. Li+ (56 mM) and veratridine (100 μM) had significantly less effect (8-15% and 15-30%, respectively) on releasing [3H]taurine compared to the K+-evoked release. 4-Aminopyridine (1 mM) had no effect on the release of [3H]taurine. The spontaneous release of [3H]taurine was also Ca2+-independent. When Na+ was omitted from the incubation medium K+-evoked [3H]taurine release was inhibited by approximately 40% at the first 5 minute depolarization period but was not affected at a second subsequent 5 minute depolarization period. The spontaneous release of [3H]taurine was inhibited by 60% in the absence of Na+. Substitution of Br− for Cl− had no effect on the release of either spontaneous or K+-evoked [3H]taurine release. However, substitution of the Cl− with acetate, isethionate, or gluconate decreased K+-evoked [3H]taurine release. Addition of taurine to the superfusion medium (homoexchange) resulted in no significant increase in [3H]taurine efflux. The taurine-transport inhibitor guanidinoethanesulfonic acid increased the spontaneous release of [3H]taurine by approximately 40%. These results suggest that the taurine release of [3H]taurine is not simply a reversal of the carrier-mediated uptake system. It also appears that taurine is not released from vesicles within the synaptosomes but does not rule out the possibility that taurine is a neurotransmitter. The data involving chloride substitution with permeant and impermeant anions support the concept that the major portion of [3H]taurine release is due to an osmoregulatory action of taurine while depolarization accounts for only a small portion of [3H]taurine release.

KEY WORDS: Taurine; taurine release; rat retina; K+ stimulation; Ca2+ independence; P2 subcellular fraction.

INTRODUCTION

It is well documented that all mammalian tissues including the retina contain high concentrations of taurine (1,2). It has also been established in the last 15 years that taurine is a necessary component of the visual system in a variety of species, including the cat (3,4), rat (5,6), monkey (7), and man (8). However, while pathologies due to taurine deficiency have been recorded, the exact mechanism as to the function of taurine is unknown (for review see ref. 9). There is considerable speculation that in brain and retinal tissues taurine may be a neurotransmitter or neuromodulator (10,11), a modifier of membrane structure and function (12), a membrane stabilizer due to its interaction with membrane phospholipids (13,14), or a modulator of Ca2+ fluxes (15-18) perhaps by regulation of protein phosphorylation (19-25).

Various criteria have been proposed to serve as an
aid in the classification of a substance as a neurotransmitter (26). Three of these criteria are that the substance has specific receptors with which it interacts, that there be specific inactivating mechanisms, and that the stimulation of neuronal afferents or elements evoke a release of the substance. Evidence of a neuroactive role for taurine in the retina is the demonstration of two binding proteins specific for taurine (27,28) and the presence of high- and low-affinity transport systems (29,30). Thus, the present study was undertaken to address the third criteria involving presynaptic release of taurine. Spontaneous and evoked efflux of \(^{3}H\)taurine from a \(P_2\) subcellular fraction of the rat retina was measured using a superfusion technique. The evoked release of \(^{3}H\)taurine was characterized by utilizing four depolarizing agents: \(K^+\), \(Li^+\), veratridine, and 4-aminopyridine. Both spontaneous and evoked release of \(^{3}H\)taurine were measured in the presence and absence of \(Ca^{2+}\), \(Na^+\) and \(Cl^-\). The present study also examined the effects of adding taurine and guanidinoethanesulfonic acid to the superfusion medium.

### EXPERIMENTAL PROCEDURE

**Materials.** 1,2-[\(^{3}H\)Taurine (20.9 Ci/mmol) was purchased from New England Nuclear. Veratridine and 4-aminopyridine were obtained from Sigma Chemical Co.

**Preparation of the Retinal \(P_2\) Subcellular Fraction.** Young adult Wistar rats (weighing 120-150g) which were fed Purina rat chow and water ad libitum, were used in all experiments. The animals were housed in the vivarium and placed on a 12-h light-dark cycle (light cycle starting at 7:00 a.m.). In the following experiments all animals were killed between 9:00 and 10:00 a.m. by anesthetizing them with water ad libitum, were used in all experiments. The animals were housed in the vivarium and placed on a 12-h light-dark cycle (light cycle starting at 7:00 a.m.). In the following experiments all animals were killed between 9:00 and 10:00 a.m. by anesthetizing them with ether and then decapitation. The eyes were immediately removed from the animals and placed in ice-cold 0.32 M sucrose. In all subsequent procedures, the retinal tissue was maintained at 2\(^\circ\)C except for the final incubation. The retinal tissue was removed from the eye cup and placed in 0.32 M sucrose at 2\(^\circ\)C. Fractionation of the retina into subcellular components was performed as described by Redburn and Thomas (31). Briefly, the retinas were gently vortexed in 0.32 M sucrose and allowed to set on ice for 7 minutes. The supernatant containing the rod outer segments was decanted and discarded. This procedure was performed twice. The remaining retinal fragments were homogenized in a Potter-Elvehjem homogenizer with 10 up and down strokes and then centrifuged for 10 min at 150 g to form a pre-\(P_2\) pellet which contains cell debris and nuclei. A \(P_2\) fraction containing photoreceptor cell synaptosomes was obtained by centrifuging the above supernatant for 10 min at 600 g. The \(P_1\) pellet was discarded and the \(P_2\) supernatant was centrifuged for 12 min at 22,000 g to produce a \(P_2\) pellet consisting of conventional synaptosomes plus mitochondria and microsomes. The \(P_2\) pellet was suspended in Krebs-Ringer (KR) medium, pH 7.4 (containing \(NaCl\), 128 mM; \(KCl\), 5 mM; \(CaCl_2\), 2.7 mM; \(MgCl_2\), 1.22 mM; \(Tris-HCl\), 15; and glucose, 10 mM) and recentrifuged. The pellet was then reinserted in Krebs-Ringer buffer by passage through a 25 gauge needle.

**Taurine Uptake System.** The incubation system contained the above KR medium and 1 \(\mu M\) \(^{3}H\)taurine (10 \(\mu C\)). The reaction mixture (0.5 ml) was preincubated for 2 min at 37\(^\circ\)C in glass test tubes treated with Prosil-28, a surface-treating agent for preparation of a water repellent surface. The reaction was initiated with the retinal preparation (~ 0.5 mg) and incubated for 30 min. The retinal \(P_2\) preparation (0.5 mg) contained less than 5 nmol of endogenous taurine. The reaction was terminated by adding 3 ml of ice-cold KR medium to the incubation system and filtering on a Millipore vacuum manifold using Whatman GF/B glass fiber filters. The filter was washed 3 times with 3 ml of the above buffer.

**Taurine Release Assay.** The moist filters containing the titrated taurine-loaded retinal homogenate from the taurine-uptake system were transferred to incubation chambers (32) and 1.4 ml of KR medium were added to the chambers. The chambers (kept at 37\(^\circ\)C in a water bath) were capped and the filters superfused with KR medium (37\(^\circ\)C, 0.5 ml/min). Two-min fractions of the superfusates were collected directly into liquid scintillation vials.

For the experiments designed to measure the evoked release of \(^{3}H\)taurine, the original KR medium was rapidly changed at the indicated times (25 and 50 min) to depolarizing media containing either elevated \(K^+\), \(Li^+\), veratridine, or 4-aminopyridine. The retinal preparation contained on the filters was superfused with the new depolarizing medium for 5 min and then superfusion was continued with the original KR medium.

When the omission of \(Ca^{2+}\) was tested in the taurine-release experiments, the KR buffer utilized for both \(^{3}H\)taurine uptake and release did not contain \(Ca^{2+}\); 2 mM EGTA was added to the KR buffer. In all the experiments in which the effects of omission of either \(Na^+\) or \(Cl^-\) were tested on \(^{3}H\)taurine release, these ions were replaced in the KR buffer with either choline (chloride) or (sodium) bromide, acetate, isethionate, or gluconate during the superfusion phase of the experiment. MgCl\(_2\) and CaCl\(_2\) were replaced with MgSO\(_4\) and CaSO\(_4\) while Tris-HCl was replaced with Tris-Base. In all experiments the isosmolarity of the KR buffer during the evoked release of \(^{3}H\)taurine was maintained. This was accomplished by reducing the \(Na^+\) concentration.

**Protein Estimation.** Protein concentrations were determined by the method of Lowry et al. (33) with bovine serum albumin utilized as the standard.

**Data Analysis.** Release of \(^{3}H\)taurine is expressed as a fractional rate vs superfusion time. Fractional rate is defined as the radioactivity released in each 2-min fraction divided by the radioactivity remaining in the retinal preparation in the preceding 2-min fraction (34). Rate constants for spontaneous efflux were calculated as negative slopes for the regression lines of the logarithm of the fractional rate.

### RESULTS

**Effect of Known Depolarizing Agents on the Spontaneous and Evoked Release of \(^{3}H\)Taurine from a \(P_2\) Retinal Fraction.** The spontaneous and \(K^+\)-evoked release of \(^{3}H\)taurine are shown in Figure 1A. Potassium chloride (56 mM) added for 5 min to the KR buffer at 25 and 50 min was used to stimulate the release of \(^{3}H\)taurine. Figure 1B and 1C show similar single experiments in which \(Li^+\) (56 mM), veratridine (100 \(\mu M\)), and 4-aminopyridine (10 mM) were used as depolarizing agents. The data were quantitated by measuring the area