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Effect of taurine on release of ^{3}H-GABA by depolarizing stimuli from superfused slices of rat brain cerebral cortex in vitro

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The effect of taurine on potassium (30 mM), veratrine (10 μg ml^{-1}) and ouabain (100 μM) induced release of ^{3}H-GABA from superfused slices of rat brain cerebral cortex has been studied. Taurine (10–100 mM) potentiated K^+-induced release of ^{3}H-GABA but was more potent in potentiating veratrine- and ouabain-induced release, having significant effects at 5 mM and 1 mM respectively. The potentiation of stimulus induced release does not appear to be due to inhibition of reuptake and the effect is consistent with both a 'modulatory' role and anticonvulsant action for taurine.

Taurine (2-aminoethane sulphonic acid) is found in high concentrations in the c.n.s. ranging from 110 mM in the neurohypophysis (Crabai et al 1974), to 8 mM in cerebral cortex (Kaczmarek 1976), and taurine is the most abundant amino acid in brains of foetal and developing animals (see Sturman & Gaull 1976). It has been suggested that taurine may be a 'modulator' of nervous transmission in the c.n.s. (Barbeau et al 1975) although previous studies with taurine at 5 mM (Crnic et al 1973) have not demonstrated any effect on basal efflux of ^{3}H-GABA. The present study was undertaken to examine the effect of taurine on stimulus evoked release of ^{3}H-GABA from superfused slices of rat brain cerebral cortex in vitro.

MATERIALS AND METHODS

Adult male Wistar rats supplied by Charles River (200–250 g) were killed by cervical fracture, the brain rapidly removed and 0·4 mm thick slices of cerebral cortex prepared using a McIlwain tissue chopper. The slices were incubated for 30 min at 37°C in 10 ml of Tyrode medium (gassed continuously with 5% CO_2 in oxygen and containing 10 μM aminooxyacetic acid (AOAA) to inhibit GABA metabolism; Wallach 1961), and ^{3}H-GABA at 1 μCi ml^{-1} (10^{-7}–10^{-8} M). After preloading, slices were washed and two were randomly allocated to each well of a 5 chamber superfusion apparatus (volume of well = 0·25 ml) and superfused with medium at 37°C at 1 ml min^{-1}. The wet weight of the slices was 19·4 ± 0·6 mg/slice (n = 36). Five min fractions (5 ml) were collected using a Gilson TDC 220 fraction collector. After an initial 20 min superfusion, sample collection was begun and the depolarizing stimuli (30 mM potassium chloride, 10 μg ml^{-1} veratrine or 100 μM ouabain) applied for 5 min at 20 min (S_1) and 60 min (S_2) superfusion continuing for up to 90 min. Taurine or other amino acids were applied 20 min before S_2 and maintained in the medium until the end of the experiment. One ml samples of superfusate were added to 10 ml 'Biofluor' for counting, and at the end of each experiment, the tissue was recovered, digested and counted (Dent & Johnson 1974) and the fractional rates of ^{3}H-GABA release calculated. The ratio (S_2/S_1) of the total evoked release of ^{3}H-GABA by the two stimulations was calculated after correction for basal flux.

Although AOAA is considered to inhibit GABA metabolism at 10 μM (Wallach 1961), when this was checked by t.l.c., over 90% of the ^{3}H-labelled material released on K^+ stimulation co-chromatographed with authentic GABA. The incubation medium contained (mM): NaCl 137; KCl 2·68; $CaCl_2$ 1·78; $MgCl_2$ 1·04; Na_2HPO_3 0·42; $NaHCO_3$ 11·9; D-glucose 5·5, pH 7·4. 4-Amino- [2,3-^{3}H] butyric acid (^{3}H-GABA; sp. act. 54 Ci mmol^{-1}) was obtained from Radiochemical Centre, Amersham.

RESULTS

The total amounts of ^{3}H-GABA released from the tissue stores by the three depolarizing stimuli applied for 5 min at S_1 in normal medium were: potassium (30 mM), 5·5 ± 0·2% (n = 28) veratrine (10 μg ml^{-1}), 8·7 ± 1·2% (n = 25) and ouabain (100 μM), 16·1 ± 0·9% (n = 17). Approximate times for the duration of the S_1 pulse by the three stimuli were 10, 15 and 20 min respectively. The concentration of ouabain used is that previously employed by Benjamin & Quastel (1972) in their studies on amino acid release from brain slices. Potassium-induced

release of ^{3}H-GABA was calcium-dependent. 50 μM nipecotic acid, a potent inhibitor of neuronal GABA uptake processes (Krogsgaard-Larsen & Johnston 1975), significantly increased evoked release by the S_1 potassium pulse (7·8 ± 0·6% total tissue stores released; n = 5, P <0·005).

Taurine (10–100 mM) significantly potentiated potassium-induced release of ^{3}H-GABA from cortical slices in a dose-dependent manner (Table 1) an effect readily enhanced by 50 μM nipecotic acid.

Table 1. Effect of taurine on stimulus-induced release of ^{3}H-GABA from cerebral cortex slices in vitro. When present, nipecotic acid (50 μM) was in the medium throughout the experiment. Rates of basal release were 0·1–0·16% (normal medium) and 0·28–0·34% (+50 μM nipecotic acid) of total tissue stores released min^{-1}. Values are mean ● s.e.m. of S_2/S_1 ratios, number of experiments are in brackets. Flow rate = 1 ml min^{-1}, Tyrode at 37° C + AOAA 10 μM.

P values indicate significant differences from respective controls.

Depolarizing agent	Treatment mM		S_2/S_1		(P, Student's t-test
KCl					
30 mM	Control		0·64±0·03	(13)	
	+Taurine	100	1·72±0·07	(4)	0·001
		50	1·18±0·03	(12)	0·001
		25	0·95±0·07	(7)	0·001
		10	0·85±0·10	(7)	0·025
	+Glutamate	50	0·55±0·03	(4)	NS
	+Isethionic acid	50	0·60±0·04		NS
+Nipecotic acid 50 μM	Control		0·72±0·03	(11)	
	+Taurine	50	1·73±0·10	(4)	0·001
		25	1·31±0·05	(4)	0·001
		10	0·86±0·01	(4)	0·025
Veratrine 10 μg ml^{-1}	Control		0·26±0·04	(8)	
	+Taurine	50	1·51±0·25	(6)	0·001
		5	0·47±0·07	(6)	0·020
Ouabain 100 μM	Control		0·17±0·02	(7)	
	+Taurine	50	1·18±0·07	(4)	0·001
		5	0·50±0·04	(4)	0·001
		1	0·34±0·05	(4)	0·005

Glutamate (50 mM) and isethionic acid (50 mM, the possible metabolite of taurine) did not alter potassium-induced ^{3}H-GABA release and this would tend to rule out any effect of taurine being due to changes in tonicity at the higher concentrations used. Taurine was also found to potentiate veratrine and ouabain induced release of ^{3}H-GABA, but at lower concentrations than in the potassium experiments. Direct release of ^{3}H-GABA by taurine was small with only 0·1–0·2% of tissue stores released/5 min fraction by 25 mM, 0·9%/5 min fraction for 50 mM and 2·3%/5 min fraction for 100 mM taurine. Taurine 50 mM did not potentiate ^{3}H-NA-induced release from this preparation; S_2/S_1 values for ^{3}H-NA release were 0·61 ± 0·07 (7) although these experiments were done in medium containing 10 μM pargyline, 60 μM ascorbic acid and 30 μM EDTA.

DISCUSSION

Taurine at 5 mM has no effect on the rate of basal release of ^{3}H-GABA from brain slices (Crnic et al 1973). However, using concentrations of 10–100 mM, taurine will potentiate potassium-induced release of ^{3}H-GABA both increasing and prolonging the S_2 pulse. Veratrine- and ouabain-induced release seem to be more sensitive to taurine, showing significant potentiation at 5 mM and 1 mM respectively. The mechanism of potentiation does not seem to be related to inhibition of GABA reuptake or to an exchange process, as direct release by millimolar concentrations of taurine is small and taurine is only a weak inhibitor of GABA uptake in this preparation (26% inhibition at 50 mM; data not shown). Also the potentiation of K^+-induced release was readily enhanced by nipecotic acid, a potent GABA uptake inhibitor (Krogsgaard-Larsen & Johnston 1975). The calcium dependency of the K^+-induced release of ^{3}H-GABA and the increased basal flux in the presence of nipecotic acid, suggest that ^{3}H-GABA may be released from neuronal rather than glial sites in this preparation. Since nipecotic acid only blocks neuronal uptake of GABA, it is possible that taurine may interfere with glial uptake of neuronally released ^{3}H-GABA rather than neuronal uptake.

Potassium, veratrine and ouabain cause membrane depolarization by distinctly different mechanisms. High extracellular K^+ reduces the K^+ electrochemical gradient across the membrane; veratrine and veratridine increase membrane permeability to Na^+ by a tetrodotoxin sensitive mechanism and ouabain, through inhibition of Na^+, K^+-ATPase causes intracellular accumulation of Na^+. Since taurine is more potent in potentiating veratrine- and ouabain-induced release of ^{3}H-GABA, this effect could possibly be related to an interaction of taurine with sodium (and perhaps related calcium fluxes) and this hypothesis is currently under investigation.

Although it may be speculative as to whether the effects on GABA release seen at millimolar concentrations of taurine are physiologically significant, taurine is present in high concentrations in synaptosomes, synaptic vesicles and mitochondria (Agrawal et al 1971; De Belleroche & Bradford 1973; Lombardini 1976) and in view of the inefficiency of taurine transport processes (Kontro & Oja 1978), high taurine concentrations may exist in extracellular spaces.

Various authors have suggested that taurine may be a modulator of nervous transmission and there are reports on taurine interactions with both GABA uptake and efflux mechanisms (for review see Collins

1977). These results demonstrate that taurine will potentiate stimulus evoked release of ^{3}H-GABA in rat cerebral cortex slices and this is an effect which is consistent with both a modulatory role and anti-convulsant action for this amino acid.

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