Taurine Release from Brain Slices in Thioacetamide-Induced Hepatic Encephalopathy in Rats

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ABSTRACT

The possible involvement of taurine in the pathogenesis of hepatic encephalopathy was studied in rats injected with thioacetamide. Spontaneous release of exogenous labeled taurine was not affected in any brain area studied, but the potassium-stimulated release was enhanced in the striatum in thioacetamide-treated rats. High concentrations of ammonium ions also evoked greater release of taurine from striatal slices in rats with thioacetamide-induced hepatic encephalopathy.

Index Entries: Hepatic encephalopathy; taurine release; thioacetamide; potassium; ammonia; GABA; brain slices; striatum; rats.

ABBREVIATIONS

GABA: γ -aminobutyric acid
HE: hepatic encephalopathy

Hepes: N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid

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INTRODUCTION

Abnormalities in neurotransmission have been widely assumed to underlie disturbances in the function of the central nervous system in hepatic encephalopathy (HE). Increased levels of γ -aminobutyric acid (GABA) in the brain or an augmentation of inhibition mediated by GABA receptors have been suggested as causal factors in the pathogenesis of hepatic coma—the terminal stage of HE (Blitzer et al., 1978; Pappas et al., 1984; Zieve, 1987). However, in some recent studies very slight changes, if any at all, were found in the brain levels of GABA in HE, indicating only minor involvement of GABA (Butterworth and Giguere, 1986; Butterworth et al., 1987). This prompted us to focus on the possible involvement of taurine in HE. This sulfur-containing amino acid abounds in the brain, is structurally related to GABA, and shares some of its inhibitory properties. Taurine is particularly enriched in synaptic structures (De Belleruche and Bradford, 1973; Kontro et al., 1980; Huxtable, 1982; Walz and Allen, 1987) and has been considered an inhibitory neuromodulator (Cooper et al., 1982; Oja and Kontro, 1983; Kontro et al., 1984; Sakai et al., 1985).

Ammonia is a main factor proposed to be involved in the pathomechanism of HE (Zieve, 1987). The concentration of ammonia is elevated in the blood and central nervous system of most patients with clinical HE and animals with experimental HE (Butterworth et al., 1987). It has been shown that ammonia can affect the membrane potential and excitability of neurons (see Cooper and Plum, 1987) and astrocytic transport of amino acid neurotransmitters (Norenberg et al., 1985), although the mechanism of the toxic effect of ammonia is not wholly established. We analyzed changes in the potassium-evoked release of taurine from various structures of the brain in rats with thioacetamide-induced HE. The striatum appeared to be the most sensitive brain area in HE and hence the effects of different concentrations of ammonium chloride on the release of taurine from striatal slices were assessed as well.

MATERIALS AND METHODS

Thioacetamide (300 mg/kg) was daily injected intraperitoneally into adult male Wistar rats (200 g) on three successive days in order to induce a precomatose stage. This hepatotoxic model of subacute hepatic encephalopathy is accompanied by elevated blood and brain ammonia, increased blood phenols and changes in other biochemical and physiological parameters characteristic of HE (Hilgier et al., 1983; Albrecht and Hilgier, 1984; Hilgier et al., 1985; Pluta and Albrecht, 1984, see Albrecht et al., 1988 for further references).

Slices 0.5 mm thick were manually cut with a tissue slicer of Stadie-Riggs type from the cerebral cortex, striatum, cerebellum, and pons. The

slices were preincubated for 30 min with 10 μM [^3H]taurine in standard Krebs-Ringer-Hepes medium (in mM; NaCl 127, KCl 5, CaCl_2 0.75, MgSO_4 1.3, Na_2HPO_4 1.3, Hepes 15, NaOH 11, D-glucose 10, pH 7.4) at 37°C under O_2 . They were then rinsed with nonradioactive medium and finally superfused at a rate of 0.25 mL/min for 50 min at 37°C in a system in which freely floating agitated slices were kept in a small volume (0.25 mL) of preoxygenated medium. This method has been shown to ensure sufficient thorough oxygenation and to preserve the viability of the preparations, as reflected by maintenance of normal ion gradients, structural integrity, and characteristics of transmitter release (Korpi and Oja, 1983, 1984; Pitkänen et al., 1985). The effluent samples were pooled during the first 20 min and subsequent 2-min fractions (0.5 mL) were collected into scintillation vials with a fraction collector (Gilson TDC 220). At 30 min, the standard superfusion medium was abruptly changed to another medium containing 50 mM K^+ ions (stimulated efflux) or left unchanged (spontaneous efflux). Striatal slices from both control and thioacetamide-treated rats were also subjected to NH_4Cl (NH_4^+ ions) at concentrations of 0.5, 10, or 50 mM; in some experiments in medium supplemented with 50 mM K^+ . The cationic balance was then obtained by equimolar reduction of Na^+ . The radioactivity in the slices and the 2-min fractions of superfusion medium was assayed by scintillation spectrometry.

The efflux rate constants of taurine for the time intervals of 20-30 min (k_1) and 34-50 min (k_2) were computed as negative slopes of the linear regression lines fitted to the logarithm of the radioactivity remaining in the slices vs superfusion time (Korpi and Oja, 1979). The constants k_2 are given as percentages of the corresponding k_1 values for each slice in order to minimize interexperimental variation. The statistically significant differences were detected by analysis of variance. As an *a priori* test, the variance ratios for the corresponding control and thioacetamide-treated groups were calculated and compared to the theoretical 5 and 1 percentage points.

RESULTS

The spontaneous efflux of taurine from the slices of all brain structures studied was slow, about 97-98% of the initial radioactivity remaining in the slices after superfusion. Potassium stimulation (50 mM K^+) from 30 min onwards induced a slow but fairly stable release of taurine in the cerebral cortex, pons (data not shown) and striatum (Fig. 1). The stimulation was only small in magnitude in the cerebellum. The range of average potassium stimulation of taurine efflux was from 1.15 to 1.85-fold in control rats when compared to spontaneous release during the same superfusion period (Table 1). Thioacetamide-induced HE did not change the basal release of taurine. The potassium-stimulated release of taurine was enhanced only in the striatum in HE (Table 1).

Fig. 1. Taurine release from striatal slices from control (○) and thioacetamide-treated (●) rats. Experimental details as in Table 1. The period of potassium stimulation (50 mM) is indicated by the bars. Individual representative experiments are shown.

The efflux rate constants (k_2) for taurine in striatal slices from control rats increased proportionally to the concentration of ammonium ions in superfusion medium, being 209% at the 50-mM NH_4^+ concentration (Table 2). The response to the combined effect of the 50-mM concentration of both K^+ and NH_4^+ was significantly less pronounced. The stimulation of taurine release by ammonium ions in thioacetamide-treated rats was similarly dose-dependent and even more pronounced than in control rats. Also in the treated rats the responses to the combination of high concentrations of K^+ and NH_4^+ ions were greatly attenuated when compared to those elicited by NH_4^+ or K^+ ions alone.

DISCUSSION

The present results show that the spontaneous efflux of taurine is not affected in HE in any of the brain structures studied. The magnitude of potassium-stimulated release varied in different brain areas, being smallest in the cerebellum, as noted in earlier reports (Okamoto and Namina, 1978; Korpi et al., 1981). The explanation why an increase in potassium-stimulated release of taurine was only discernible in striatal

Table 1
Fractional Efflux Rate Constants of Taurine in Rat Brain Slices

Type of release	Control rats			Thioacetamide-treated rats		
	k_1 ($\times 10^{-3} \text{ min}^{-1}$)	k_2 (% of k_1)		k_1 ($\times 10^{-3} \text{ min}^{-1}$)	k_2 (% of k_1)	
<u>Cortex</u>						
Spontaneous	1.477 ± 0.188	83.4 ± 3.1	(6)	1.888 ± 0.344	88.4 ± 1.9	(3)
Stimulated	1.666 ± 0.116	149.9 ± 5.7	(13)	1.725 ± 0.179	148.4 ± 9.0	(7)
<u>Cerebellum</u>						
Spontaneous	2.319 ± 0.058	77.8 ± 1.1	(3)	2.235 ± 0.699	77.4 ± 1.5	(4)
Stimulated	1.555 ± 0.094	89.4 ± 1.5	(8)	1.852 ± 0.172	97.7 ± 5.5	(8)
<u>Striatum</u>						
Spontaneous	1.060 ± 0.095	83.4 ± 1.7	(4)	1.304 ± 0.416	89.5 ± 4.8	(3)
Stimulated	0.898 ± 0.166	155.0 ± 5.4	(8)	0.971 ± 0.074	$173.5 \pm 5.1^*$	(6)
<u>Pons</u>						
Spontaneous	1.892	75.5	(2)	2.391	75.5	(2)
Stimulated	1.883 ± 0.271	133.3 ± 5.3	(5)	2.107 ± 0.262	119.8 ± 5.5	(5)

The slices were preloaded with $10 \mu\text{M}$ [^3H]taurine for 30 min at 37°C in Krebs-Ringer-Hepes medium (pH 7.4) and then superfused for 50 min with the same medium. At 30 min, 50 mM Na^+ ions were replaced by 50 mM K^+ ions (stimulated release) or the medium was left unchanged (spontaneous release). The rate constants k_1 refer to the superfusion period of 20–30 min and the constants k_2 to the period of 34–50 min. Number of experiments in parentheses. Statistically significant difference from the control: $*p < 0.05$.

Table 2
Effect of Ammonium Ions on the Fractional Efflux Rate Constants of Taurine from Rat Striatal Slices

Substance present from 30 to 50 min (mM)	Control rats		Thioacetamide-treated rats	
	k_2 (% of k_1)		k_2 (% of k_1)	
NH_4^+ 0.5	73.8 ± 2.9	(5)	78.9 ± 3.9	(5)
NH_4^+ 10	121.9 ± 4.4	(4)	132.4 ± 2.3	(4)
NH_4^+ 50	209.9 ± 23.6	(10)	$323.0 \pm 30.6^{**}$	(9)
NH_4^+ 50 + K^+ 50	134.2 ± 10.4	(10)	$239.3 \pm 35.7^{**}$	(5)
NH_4^+ 10 + K^+ 50	110.0 ± 6.6	(5)	115.2 ± 8.1	(5)
NH_4^+ 10 + K^+ 50 ^a	124.1 ± 2.8	(5)	121.5 ± 10.5	(10)
K^+ 50	155.0 ± 5.4	(8)	$173.5 \pm 5.1^*$	(6)

^a NH_4^+ was present from the beginning of the superfusion.

Statistically significant differences from the corresponding control: $*p < 0.05$, $**p < 0.01$.

slices in HE is probably rather complex owing to mutual interactions of both excitatory and inhibitory neurotransmitters. Taurine is not generally considered a classical transmitter, but mounting evidence suggests that it may interfere with the function of the inhibitory neurotransmitter GABA (Namima et al., 1983; Malminen and Kontro, 1986; Kontro and Oja, 1990) and may thence affect neuronal excitability in HE. Although studies on

the contribution of GABA to the pathogenesis of HE have yielded rather controversial data, a recent study has indicated a significant inhibition of potassium-stimulated release of [^3H]GABA in striatal synaptosomes from rats in carbon tetrachloride-induced hepatic coma (Diaz-Munoz and Tapia, 1988). The simultaneously observed enhancement in the uptake of GABA should cause a decrease in the efficacy of the inhibitory function of GABA. The potentiation of potassium-evoked release of taurine may result in increased occupation of synaptic receptor sites of GABA by taurine. This appears plausible in the light of evidence available that taurine interferes with the binding of GABA to both postsynaptic GABA_A and GABA_B receptors (Greenlee et al., 1978; Hitzemann and Loh, 1978; Kontro and Oja, 1990) and may modulate the function of presynaptic GABA autoreceptors (Namima et al., 1983; Medina and De Robertis, 1984; Malminen and Kontro, 1986). Moreover, it has been recently shown that in HE taurine is increased to levels which *in vitro* markedly inhibit the binding of GABA (Maddison et al., 1990).

The characteristic morphological changes indicate that astrocytes are the cell type primarily affected in HE (Norenberg, 1981). In chronic liver diseases, such as HE, the protoplasmic astrocytes increase in number and size, taking on a characteristic appearance identified as the Alzheimer Type II astrocyte with an enlarged nucleus and a thin rim of surrounding cytoplasm (Zieve, 1982). The astrocytes show likewise a whole spectrum of biochemical abnormalities, such as changes in amino acid neurotransmitters and in transport of mono- and divalent ions (see Albrecht et al., 1988). Furthermore, swelling of astrocytes is a salient feature in a number of pathological conditions, HE included (Kimmelberg and Ransom, 1986). Potassium stimulation also increases the cell volume, this being accompanied by the release of taurine (Pasantes-Morales and Schousboe, 1989; Martin et al., 1990). It is thus conceivable that striatal astrocytes contribute to the increase in stimulated release of taurine in HE. Striatal astrocytes may also be more sensitive to swelling in HE and release more taurine in high-potassium media. Pertinent to the aforementioned discussion are observations that release of taurine can be elicited from astrocytes by activation of their β -adrenergic receptors (Van Calker and Hamprecht, 1981; Shain et al., 1986). An activation of β -adrenergic receptors in response to swelling of cerebral cortex slices (Bourke et al., 1983) and ammonia-induced aberration of the adrenergic cAMP-system in primary astrocytes (Lisowsky et al., 1986) suggest that ammonia in HE may affect the volume regulation of astrocytes and increase the release of taurine from striatal slices *in vitro*.

We cannot rule out the possibility that the neuronal elements in the striatum are also affected in HE, although likely not so much as the glial cells. Dopaminergic axons make direct synaptic connections with GABAergic neurons in the rat striatum (Kubota et al., 1987). The striatal release of GABA may be differentially regulated by D₁ and D₂ receptors, the latter being responsible for inhibitory modulation (Reid et al., 1990).

Even low concentrations of ammonia evoke release of dopamine from rat cerebral synaptosomes (Erecińska et al., 1987). The increased release of taurine in HE may be compensatory to the diminished release of GABA in maintenance of synaptic inhibition in the striatum.

Electrophysiological studies have demonstrated that ammonia affects the excitability of neuronal membranes by shifting inhibitory post-synaptic potentials toward the resting potential and slowing extrusion of Cl^- from neurons (Lux et al., 1970). The effects of NH_4^+ ions also partially mimic those of K^+ or Na^+ ions (see Cooper and Plum, 1987). The attenuation of the release of taurine in the presence of both high K^+ and NH_4^+ may reflect decreased synaptic excitation (Raabe, 1989), which may be due to K^+ -like ionic properties of NH_4^+ and/or inhibition of K^+ uptake into astrocytes (Norenberg et al., 1985). A prolonged exposure to NH_4^+ results in suppression of the frequency of spontaneous action potentials, increase of Na^+ , and decrease of K^+ intracellularly in slices (Benjamin et al., 1978). The potassium-stimulated release of taurine may hence decrease in the presence of NH_4^+ . We cannot thus exclude the possibility that some of the NH_4^+ -induced release of taurine originates from neurons. The question must be settled with studies on isolated or cultured cells of glial and neuronal origins.

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