Release of [^3H] γ -Aminobutyric Acid by Taurocyamine in Rat Brain Slices

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Accepted May 25, 1984

Recently, much attention has been focused on the possible role of taurine in the central nervous system (CNS). Although anticonvulsant actions of taurine have been known, functional roles of taurine in the CNS are still undefinable (1, 2). It has been shown that guanidinoethyl sulfonate (taurocyamine), an amidino analog of taurine, is a competitive inhibitor of taurine transport in tissue preparations (3). Rats maintained on 1% taurocyamine in their drinking water showed a marked drop in the taurine content of various tissues (3). With respect to the pharmacological actions of taurocyamine in the CNS, it is known to elicit an immediate and intense seizure activity by intracerebral injection in seizure-susceptible rats (4). We also found that taurocyamine has an epileptogenic action; it caused severe hippocampal seizure activities few minutes after intracerebroventricular injection at a dose of 300 μg . However, it is not clear whether this effect of taurocyamine is related to the inhibition of taurine transport. In connection with these observations, the present study was undertaken to examine possible effects of taurocyamine on γ -aminobutyric acid (GABA) release in rat brain slices.

[^3H]GABA (57 Ci/mmol) was obtained from the Radiochemical Centre, Amersham. Taurocyamine was synthesized according to the method of Huxtable et al. (3). Male Sprague-Dawley rats, weighing about 150–200 g, were used throughout. The slices of cerebral cortex were prepared as described previously (5). Chronic treatment with taurocyamine was performed by maintaining experimental rats on drinking water containing 1% taurocyamine for 10 days; taurine

content in the cerebral cortex was decreased to about 60% by this treatment. Release of [^3H]GABA from the preloaded slices was examined in the presence of 0.1 mM aminooxy-acetic acid by the superfusion method as described elsewhere (6). GABA contents in the tissue preparations were measured by an enzymatic method (7). GABA receptor binding was examined in the crude synaptic membranes of the cerebral cortex by the method of Ticku et al. (1980) (8).

As depicted in Fig. 1A, change of the superfusion medium (Krebs-Ringer, KR) to KR containing 2 mM taurocyamine was followed by a sharp increase in the release of [^3H]GABA from the preloaded cortical slices. The enhanced release of GABA gradually returned to the normal level after rechanging the medium to normal KR, showing that the stimulatory effect of taurocyamine is reversible. In contrast, 2 mM taurine had no effect on both the spontaneous and taurocyamine-stimulated release of GABA. In addition, 2 mM taurocyamine had a stimulatory effect even in the presence of 30 mM KCl (data not shown). Taurocyamine also facilitated the release of GABA even in the absence of calcium (data not shown). Thus, the release of GABA induced by taurocyamine is qualitatively different from that by depolarization. In agreement with the previous finding (3), we found that taurocyamine at concentrations of up to 1 mM had no effect on high affinity uptake of GABA in the slices. Furthermore, the superfusion method, which was used in the present study, eliminated apparent reuptake of released [^3H]GABA as shown in Fig. 1A (dotted line). These findings

Fig. 1. Release of [^3H]GABA by taurocyamine from the preloaded slices (A) and P_2 fractions (B) of rat cerebral cortex. The preparations were loaded with $10\ \mu\text{M}$ [^3H]GABA by incubation at 37°C for 10 min and then washed with Krebs-Ringer (KR), transferred to superfusion chambers, and perfused at 37°C with KR at a constant flow rate of $2.0\ \text{ml/min}$. The effects of drugs were examined by switching to KR containing the drugs. Released radioactivity was expressed as a percentage of the total radioactivity taken-up by the slices. (A) $2\ \text{mM}$ taurocyamine ($\circ$), $2\ \text{mM}$ taurocyamine + $5\ \text{mM}$ taurine ($\bullet$), $5\ \text{mM}$ taurine ($\blacktriangle$). Apparent uptake of [^3H]GABA from the superfusion medium by slices was examined by superfusing the non-preloaded slices with $2000\ \text{d.p.m./ml}$ of [^3H]GABA as described previously (5). (dotted line). (B) $1\ \text{mM}$ taurocyamine ($\square$), $5\ \text{mM}$ taurocyamine ($\blacksquare$). Points are means for six separate experiments. The standard error of each value was less than 10%.

indicate that the marked increase of [^3H]-GABA in the perfusate is due to enhanced release, not to the inhibition of reuptake. In order to know the specificity of the stimulatory effect of taurocyamine on neurotransmitter release, we next examined the effect of the drug on the spontaneous release of [^3H]ACh and of [^3H]glutamate from the preloaded cortical slices (preloading was performed with either $0.2\ \mu\text{M}$ [^3H]choline or $10\ \mu\text{M}$ [^3H]glutamate), showing that it had no effect (data not shown). These observations suggest that taurocyamine might release GABA through the exchange. Furthermore, taurocyamine also evoked the release of GABA from the preloaded crude synaptosomal fractions (P_2) of cerebral cortex (Fig. 1B). The site of origin of GABA released by taurocyamine is not clear. Results obtained with the P_2 fractions indicated that synaptosomes at least are a site for taurocyamine-evoked release of GABA. It is also possible that taurocyamine facilitated GABA release from glial cells. This possibility was supported by the lack of calcium-dependency and the effectiveness in the depolarized state of the stimulatory effect of taurocyamine. The stimulation by taurocyamine of spontaneous release of GABA was concentration-

Fig. 2. Dose-response curve for the stimulatory effect of taurocyamine on [^3H]GABA release. * $P < 0.01$, ** $P < 0.001$, compared with the control (spontaneous release).

dependent; it facilitated the release by 2- to 4-fold at the concentrations of 1 to $10\ \text{mM}$ (Fig. 2). The amount of released GABA by $5\ \text{mM}$ taurocyamine corresponded to that induced by $30\ \text{mM}$ KCl (data not shown).

In order to characterize the stimulatory effect of taurocyamine on GABA release, we next examined the effect of the drug on the GABA system *in vivo*. After 10 days of taurocyamine-treatment, GABA contents in the cerebral cortex, cerebellum, striatum, hippocampus and hypothalamus and in crude

synaptosomal fractions of the cortex were not changed. In addition, time-dependent increase of GABA content in these regions, which were observed after blocking GABA transaminase by aminooxyacetic acid (9), were not changed by chronic taurocyamine-treatment. These results indicate there is no apparent effect of taurocyamine on the metabolism of GABA *in vivo*. To confirm this idea, we examined GABA receptor binding using [³H]GABA as a ligand in control and taurocyamine-treated rats. In agreement with the previous report by others (8), crude synaptic membranes of cerebral cortex from normal rats had two apparent binding sites for GABA. There was no change in K_d and B_{max} values between control and taurocyamine-treated groups (data not shown). Thus, taurocyamine failed to affect the GABA system *in vivo* at the doses at which tissue taurine was markedly depleted. It remains unclear as to whether the release of GABA by taurocyamine might be functional. The present study clearly showed that taurocyamine, an competitive inhibitor of taurine transport, acts as an agent for facilitating GABA release in an *in vitro* system.

Acknowledgments: This research was supported by grants from the Ministry of Education, Science and Culture of Japan in 1983 and from the Taisho Pharmaceutical Co., Ltd., Tokyo. We thank Miss Rinko Katsuda for technical assistance.

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