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EFFECTS OF ANTICONVULSANT AND CONVULSANT GAMMA-BUTYROLACTONES AND THIOBUTYROLACTONES ON GABA-MEDIATED CHLORIDE UPTAKE

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The anticonvulsant and convulsant gamma-butyrolactones (GBLs) are alkyl-substituted derivatives of lactonized gamma-hydroxybutyrate (GHB), a five-membered heterocyclic ring structure. When unsubstituted, high doses of GBL or GHB cause a trance-like encephalopathy in animals (1,2). Alpha substitution with an alkyl group produces anticonvulsants effective against pentylenetetrazol (PTZ) induced seizures (3,4). Gamma substitution alone also results in mild anticonvulsant activity (3,4). In contrast, beta substitution results in potent convulsant activity (4,5).

Substitution of sulfur for the oxygen moiety in the ring results in a marked increase in potency, though the anticonvulsant or convulsant activity remains determined by its substitution pattern (6, and unpublished data). Thus, the beta-substituted thiobutyrolactone (TBL), β-ethyl,β-methyl-thiobutyrolactone (βEMTBL), is a much more potent convulsant than its corresponding GBL, β-ethyl,β-methyl-gamma-butyrolactone (βEMGBL). In addition, we have demonstrated that α-ethyl,α-methyl-thiobutyrolactone (αEMTBL) is not only more potent than α-ethyl,α-methyl-gamma-butyrolactone (αEMGBL) against PTZ-induced seizures, but also has an increased spectrum of activity and exhibits protective activity against maximal electroshock (MES) induced seizures (6,7).

We hypothesized previously that GBLs may interact with the picrotoxinin receptor and demonstrated structural similarities to active areas of picrotoxinin using computer-assisted modeling (8). Recently, the GBLs have been shown to interact with the t-butyl-bicyclophosphorothionate (TBPS)/picrotoxinin binding site (9) in a competitive manner (10). The TBLs interacted in a similar manner (unpublished data) but possessed much higher affinities. Thus, it is plausible that the substituted GBLs and TBLs may have their actions at the TBPS/picrotoxinin site and their effects may be mediated by the GABA receptor/chloride ionophore complex.

GABA-dependent chloride ion (Cl^-) uptake has been demonstrated in simple brain microsac preparations using [^{36}Cl] and GABA or GABA agonists (11,12). This response can be diminished by picrotoxinin, correlating with its proposed interactions at the GABA receptor/chloride ionophore complex (13), and augmented by pentobarbital as demonstrated in previous studies (11,12). We have used this method to examine the effects of the anticonvulsant and convulsant GBLs and TBLs on chloride uptake and now report the results of these studies.

MATERIALS AND METHODS

αEMGBL, βEMGBL, αEMTBL and βEMTBL were synthesized as previously described (3,5,6). Adult female Sprague-Dawley rats were decapitated, their brains were removed, and cortex was quickly dissected from the brain. The tissue was homogenized by hand with 10 passes of a teflon homogenizer in ice-cold Tris/Hepes buffer (pH 7.5) and centrifuged at 1000*g* for 15 min. The supernatant fraction

was discarded, and the pellet was resuspended in buffer and centrifuged again at 1000g. The final pellet was resuspended with 6 vol. buffer per original weight of cortex for a final protein concentration of 6-7 mg/ml as determined by the method of Lowry et al. (14). The tissue was preincubated at 30° for 30 min and a 200 μl aliquot was added to a reaction medium consisting of 0.08 μCi NaCl [^{36}Cl], various concentrations of GABA, and/or the lactone tested. The assay was stopped at 4 sec with 4 ml of ice cold buffer, poured over Whatman GF/C filters in a Hoefer manifold, and washed with 8 ml buffer. The filters were counted with a Beckman LS 3800 counter. All points were performed in triplicate, with the exception of the standard GABA dose curve, which represented the collection of all experiments (N = 18 samples/point). Typical control values were 110 cpm in the absence of GABA and 220 cpm with 100 μM GABA added. Filter blanks were approximately 60 cpm. The data obtained were expressed as a percentage of the [^{36}Cl] uptake elicited by 100 μM GABA, defined as the amount of ^{36}Cl accumulated in the presence of 100 μM GABA minus the accumulation in the absence of GABA. 100 μM GABA produced maximal uptake in our experiments and those previously described (11,12). ED_{50} values ($\pm$S.E.) were calculated by probit analysis, using the SAS statistics program.

RESULTS

Our work demonstrated a significant inhibition of GABA-mediated Cl^- uptake by picrotoxinin and augmentation by pentobarbital (Fig. 1, A and B) consistent with results of previous studies (11,12). The effect of various concentrations of GABA on [^{36}Cl] uptake resulted in a sigmoidal dose-response curve with an ED_{50} of 21.1$\pm$1.6 μM, which also was similar to previous studies (11,12). No significant stimulation of uptake was obtained with the anticonvulsants, αEMGBL and αEMTBL, in the absence of GABA, nor was there supramaximal uptake of chloride at 100 μM GABA upon their additions. Likewise, there were no decrements in absolute Cl^- uptake when the convulsant beta compounds were tested in the absence of GABA.

Addition of the tested GBLs and TBLs resulted in distinct shifts in the GABA dose-response curves (Fig. 1, C-F). All of the shifts were statistically significant ($p \leq 0.02$) as determined by analysis of covariance on the probit regressions, with the exception of 1 mM αEMGBL. The anticonvulsant α-substituted compounds caused left shifts of the dose-response curves and promoted GABA-mediated chloride uptake. Addition of 1 mM αEMTBL decreased the ED_{50} to 15.0$\pm$2.6 μM. αEMGBL was less potent and showed no significant activity at 1 mM. A higher concentration of 10 mM decreased the ED_{50} to 16.2$\pm$5.2 μM.

In contrast, convulsant β-substituted compounds shifted the dose-response curves to the right and reduced chloride uptake (Fig. 1, E and F). Addition of βEMGBL at concentrations of 100 μM and 1 mM caused increases of ED_{50}s to 51.9$\pm$24.6 μM and 89.6$\pm$23.5 μM, respectively. The TBL compound was more potent than its GBL analogue; addition of 100 μM βEMTBL resulted in a marked increase of the ED_{50} to 200$\pm$103 μM.

DISCUSSION

We previously demonstrated that these compounds which differ only in their alkyl substitution and their ring heteroatom have profoundly different effects in animals as convulsants or anticonvulsants (3-6). We have also demonstrated a competitive interaction with the TBPS/picrotoxinin site (10), a member of the GABA receptor/chloride ionophore complex (15). The current data now indicate that there is a significant alteration of GABA-dependent chloride uptake by the GBLs and TBLs in a manner consistent with their in vivo activity. As no effects on chloride uptake were elicited by the GBLs and TBLs alone, we conclude that these compounds were GABA-dependent, rather than GABA-independent effectors of the chloride ionophore.

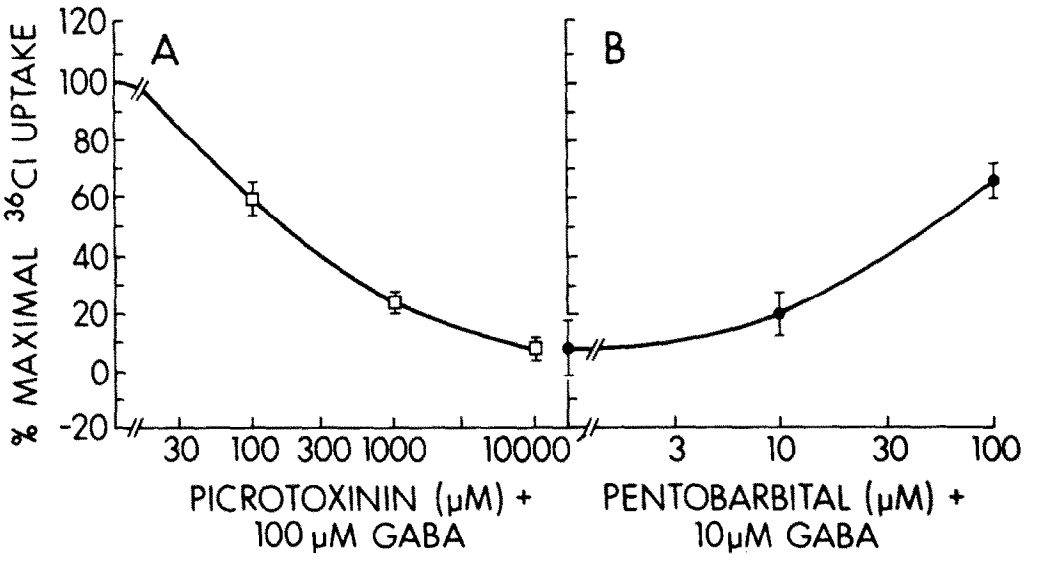

Fig. 1. (A) Picrotoxinin inhibition of chloride uptake induced by 100 μM GABA. (B) Pentobarbital augmentation of chloride uptake induced by 10 μM GABA. (Bars represent ± SD.)

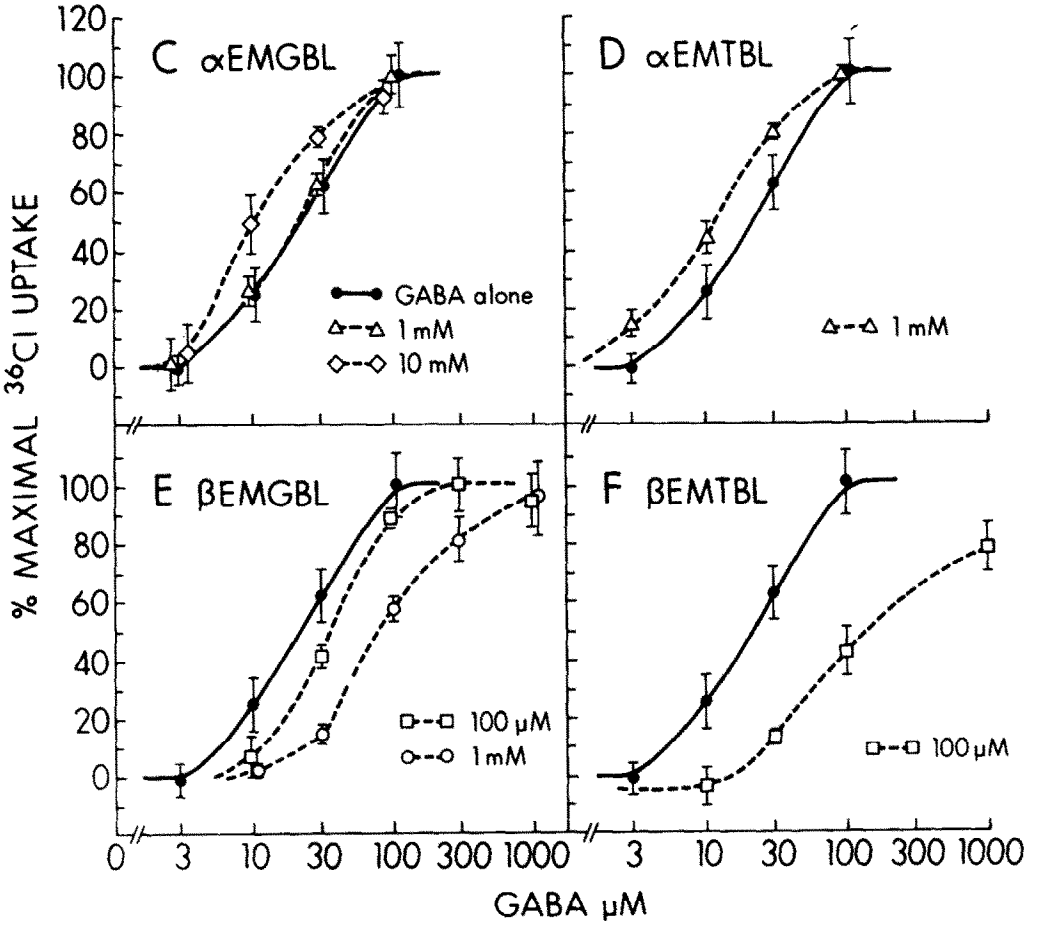

Fig. 1 (cont.). (C,D) Augmentation of GABA induced chloride uptake by the anticonvulsants αEMGBL and αEMTBL. (E,F) Inhibition of GABA induced chloride uptake by convulsants βEMGBL and βEMTBL. (100% uptake represents the accumulation of ^{36}Cl elicited by 100 μM GABA minus the accumulation in the absence of GABA. The solid line represents standard GABA dose response; bars represent ± SD.)

It is likely that the convulsant β-substituted GBLs and TBLs act at the TBPS/picrotoxinin site in a manner similar to picrotoxinin in view of their competitive interactions with the receptor, their inhibition of Cl^- uptake and their convulsant effects. The possible mechanisms of the α-substituted GBLs and TBLs have implications which are far more interesting, as they augment GABA-mediated Cl^- uptake and are anticonvulsant, in sharp contrast to the β-substituted compounds.

If the picrotoxinin receptor were capable only of inhibiting chloride uptake, the present data suggest that α-substituted anticonvulsant GBLs and TBLs may act at another unique site. Thus, interaction at the TBPS/picrotoxinin site would be fortuitous and unrelated to the anticonvulsant mechanism. Alternatively, αGBLs and αTBLs may be exerting their anticonvulsant effect by competing with an endogenous picrotoxinin-like substance at the TBPS/picrotoxinin site. However, no endogenous picrotoxinin-like convulsant has yet been identified.

It is more probable that the picrotoxinin receptor exhibits a bipotential response, and may diminish or enhance GABA-mediated chloride uptake depending on the ligand presented. Thus, the anticonvulsant α-substituted GBLs and TBLs would function as inverse agonists of the convulsants picrotoxinin, βGBLs and βTBLs; this best explains the competitive binding of the anticonvulsants and convulsants to the same receptor despite their opposite actions in vitro and in vivo, and suggests that GBLs, TBLs and picrotoxinin are modulators, rather than channel blockers of the GABA receptor/chloride ionophore complex.

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