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PRELIMINARY COMMUNICATION

CONVULSANT AND ANTI-CONVULSANT GAMMABUTYROLACTONES BIND AT THE PICROTOXININ/T-BUTYLBICYCLOPHOSPHOROTHIONATE (TBPS) RECEPTOR

Jeffrey A. Levine, James A. Ferrendelli and Douglas F. Covey

Departments of Pharmacology and Neurology
Washington University School of Medicine
St. Louis, Mo. 63110, U.S.A.

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Alkyl substituted gammabutyrolactones (GBL's) are known (1-3) to be potent neuropharmacologic agents, the activities of which are dependent on the substitution pattern. Beta-alkyl substituted GBL's, such as β-ethyl-β-methyl GBL (β-EMGBL), cause seizures in experimental animals similar to those produced by picrotoxinin (PTXN), whereas α-alkyl substituted GBL's, such as α-ethyl-α-methyl GBL (α-EMGBL), inhibit seizures induced by PTXN, pentylenetetrazole, or β-substituted GBL's. Structure-activity studies combined with computer modelling led Klunk *et al.* (4,5) to suggest that both the convulsant and anticonvulsant GBL's, as well as picrotoxinin and related convulsants, all act at a common molecular receptor site. In this paper we will present data demonstrating that both the convulsant β-EMGBL and the anticonvulsant α-EMGBL bind to the putative picrotoxinin receptor and discuss evidence that they do indeed express their effects via this receptor.

Utilizing [^{35}S]-t-butylbicyclophosphorothionate ([^{35}S]-TBPS) as a probe in receptor binding assays, Squires and co-workers (6,7) have demonstrated that PTXN and TBPS bind at either a common receptor or at overlapping receptor sites. This binding site is believed to be associated with a γ-aminobutyric acid regulated Cl^- channel (8,9). A number of convulsant drugs have been shown to bind at this receptor (6), and there is a correlation between the binding affinities and potencies of these convulsant drugs which suggests that they may mediate their effects via this receptor. Very little data concerning binding of anticonvulsant drugs at this receptor has been reported and one can only speculate about their sites of actions.

MATERIALS AND METHODS

α-EMGBL and β-EMGBL were prepared as described previously (1,2). Unlabeled and [^{35}S]-labeled TBPS (sp. act. 60-65 Ci/mmole at beginning of study, adjusted for decay thereafter) were obtained from New England Nuclear Company (Boston, MA).

Cerebral hemispheres were dissected from male Sprague-Dawley rats and the $P_2 + P_3$ membrane fraction prepared as described by Trifiletti *et al.* (10). The membrane suspensions were stored frozen at -70° and thawed and re-suspended with a Polytron

immediately prior to use. Protein was determined using the Bio-Rad Protein Assay Kit I (Richmond, CA) in the presence of 0.005% Triton X-100.

For binding assays, 100 µl of tissue homogenate was mixed with 50 µl of [^{35}S]-TBPS at the appropriate concentration in 1 M NaBr and 50 µl of 50 mM Tris-citrate buffer (pH 7.5 at 0°) containing either no additive or the appropriate concentration of drug. For the inhibition experiments, final [^{35}S]-TBPS concentration was 2 nM. For the saturation analysis experiments, the specific activity was diluted 10-fold with unlabeled TBPS; concentrations of [^{35}S]-TBPS ranging between 1 and 60 nM were used. The samples were incubated for 90 min at 25°, diluted with 3 ml of 0.9% NaCl, filtered through Whatman GF/B microfiber filter discs using slight vacuum, and washed twice with 3 ml of 0.9% NaCl. The filters were dried and then counted by conventional liquid scintillation counting. Non-specific binding was defined as that observed in the presence of 10 µM unlabeled TBPS.

RESULTS AND DISCUSSION

In confirmation of the work of other investigators (6,7,10), we observed saturable binding of [^{35}S]-TBPS with K_d=25±1 nM and B_{max}=1.7±0.1 pmole/mg-protein. We found that both α-EMGBL and β-EMGBL inhibit the binding of TBPS in a concentration-dependent fashion (fig. 1). From the inhibition curves, the IC_{50} can be estimated to be approximately 2.5 mM and 100 µM, respectively. To determine the nature of this inhibition the concentration dependence of TBPS binding was studied in the presence of single concentrations of either α-EMGBL or β-EMGBL. Scatchard analysis of the data (fig. 2) shows that both α-EMGBL and β-EMGBL are competitive inhibitors of TBPS binding, increasing the apparent K_d without altering the B_{max}.

That both the convulsant and the anti-convulsant GBL's bind competitively to the PTXN/TBPS binding site suggests, but does not prove, that they both act at this site. We do, however, have additional evidence to support this view. Pharmacological studies by Klunk et al. (2) showed that, in mice, PTXN and β-EMGBL have similar pharmacological profiles and display parallel dose-response curves. Pre-treatment of the animals with α-EMGBL resulted in parallel shifts of the same magnitude in the dose-response curves for both PTXN and β-EMGBL. These results suggest the same site of action for all three drugs.

Correlation of the IC_{50}'s and the potencies (LD_{50}) of a number of convulsant drugs (including β EMGBL) believed to have mechanisms similar to PTXN suggests that the PTXN/TBPS binding site is indeed involved in the action of these drugs. β-EMGBL is less potent than picrotin, is approximately the same as bemegride, and is more potent than pentylenetetrazole. Their IC_{50}'s are 12, 130, and 560 µM respectively (6). The relatively poor affinity of the anticonvulsant α-EMGBL (IC_{50} of 2.5 mM vs 100 µM for β-EMGBL) may be a reflection of the low potency of α-EMGBL. A dose of about 500 mg/kg of α-EMGBL is required to fully block the seizures caused by 50 mg/kg of β-EMGBL. We are in the process of investigating the correlation between anticonvulsant binding and potency by attempting to prepare high-affinity anti-convulsant GBL's.

We anticipate that the binding assay described here will prove complementary to animal studies in defining drug potency. Although the binding assay will not distinguish between convulsant and anticonvulsant drugs acting at the PTXN/TBPS receptor, it will enable one to better model the molecular receptor and thereby allow design of molecules for best receptor fit unencumbered by the ambiguities involved in whole animal studies.

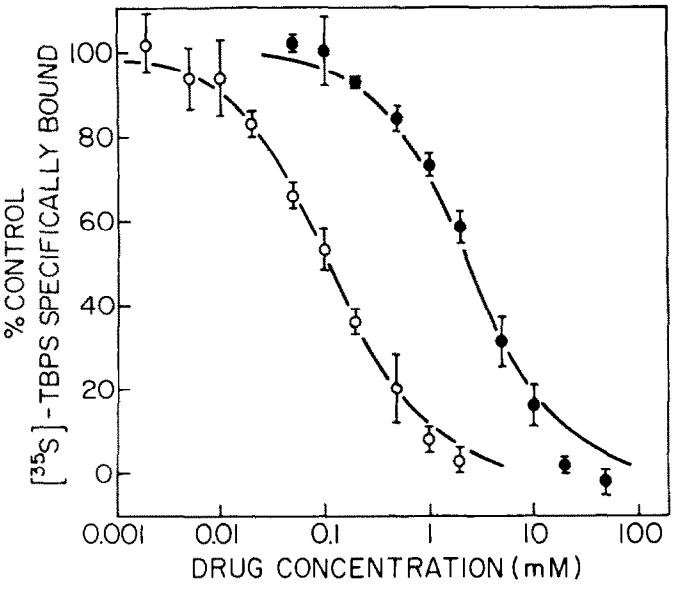

Fig. 1. Inhibition of specific [^{35}S]-TBPS binding to rat cerebral membranes by α-EMGBL (●) or β-EMGBL (○). The points represent means ± S.D. of three experiments, each done in duplicate.

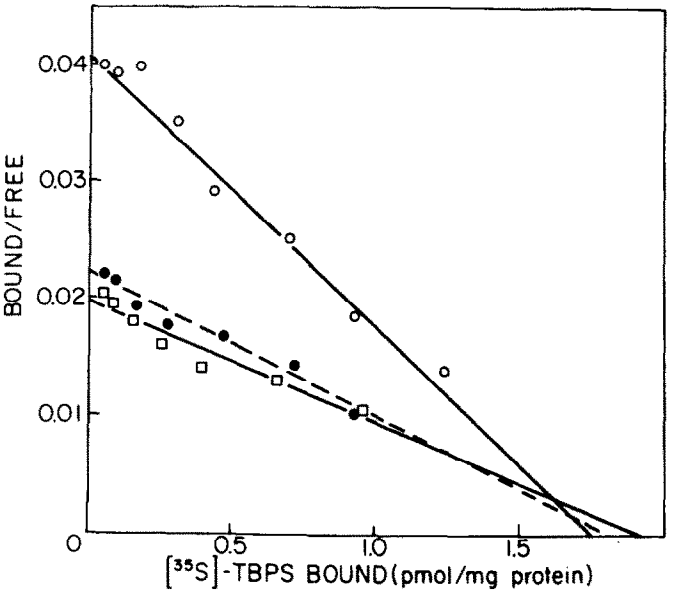

Fig. 2. Scatchard analysis of [^{35}S]-TBPS binding to rat cerebral membranes. Points are from a representative experiment. Lines are unweighted least squares lines. The experiment was performed three times in duplicate with similar results. Key: [^{35}S]-TBPS saturation in the presence of no added drug (○), 2.5 mM α-EMGBL (●), or 100 μM β-EMGBL (□). With no drug, K_d=25±1 nM , B_{max}=1.7±0.1 pmole/mg-protein; with 2.5 mM α-EMGBL, K_d=54±8 nM, B_{max}=1.6±0.2 pmole/mg-protein; with 100 μM β-EMGBL, K_d=56±9 nM, B_{max}=1.7±0.2 pmole/mg-protein.

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