Molecular Pharmacology
molpharm.aspetjournals.org

Molecular Pharmacology **November 1988** vol. 34 no. 5 **605-613**

Determination and characterization of a cannabinoid receptor in rat brain.

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Abstract

The determination and characterization of a cannabinoid receptor from brain are reported. A biologically active bicyclic cannabinoid analgetic CP-55,940 was tritium-labeled to high specific activity. Conditions for binding to rat brain P2 membranes and synaptosomes were established. The pH optimum was between 7 and 8, and specific binding could be eliminated by heating the membranes to 60 degrees. Binding to the P2 membranes was linear within the range of 10 to 50 micrograms of protein/ml. Specific binding (defined as total binding displaced by 1 microM delta 9-tetrahydrocannabinol (delta 9-THC) or 100 nM desacetyllevonantradol) was saturable. The Kd determined from Scatchard analysis was 133 pM, and the Bmax for rat cortical P2 membranes was 1.85 pmol/mg of protein. The Hill coefficient for [3H]CP-55,940 approximated 1, indicating that, under the conditions of assay, a single class of binding sites was determined that did not exhibit cooperativity. The binding was rapid (kon approximately 2.6 x 10(-4) pM-1 min-1) and reversible (Koff approximately 0.016 min-1) and (koff' greater than 0.06 min-1). The two Kd values estimated from the kinetic constants approximately 55 pM and exceeded 200 pM, respectively. The binding of the agonist ligand [3H]CP-55,940 was decreased by the nonhydrolyzable GTP analog guanylylimidodiphosphate. The guanine nucleotide induced a more rapid dissociation of the ligand from the binding site, consistent with an allosteric regulation of the putative receptor by a G protein. The binding was also sensitive to MgCl2 and CaCl2. Binding of [3H]CP-55,940 was displaced by cannabinoid drugs in the following order of potency: CP-55,940 greater than or equal to desacetyllevonantradol greater than 11-OH-delta 9-THC = delta 9-THC greater than cannabinol. Cannabidiol and cannabigerol displaced [3H]CP-55,940 by less than 50% at 1 microM concentrations. The (-)-isomer of CP-55,940 displaced with 50-fold greater potency than the (+)-isomer. This pharmacology is comparable to both the inhibition of adenylate cyclase in vitro and the analgetic activity of these compounds in vivo. The criteria for a high affinity, stereoselective, pharmacologically distinct cannabinoid receptor in brain tissue have been fulfilled.

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