Rapid communication

EVIDENCE FOR SPECIFIC METHYLENEDIOXYMETHAMPHETAMINE (ECSTASY) BINDING SITES IN THE RAT BRAIN

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Methylenedioxymethamphetamine (MDMA) is a psychotropic agent which is chemically related to both amphetamine-like stimulants and hallucinogens such as mescaline (Shulgin and Nichols, 1978). When ingested, this compound has been reported to produce a unique feeling of well being without hallucination (Anderson et al., 1978; Dowling et al., 1985). These properties have made MDMA a useful adjunct to psychotherapy. Recently, MDMA has found wide acceptance as a purported safe recreational drug. However, very little research has been performed on the mechanism of action or potential side effects. Due to the rapidly expanding use of MDMA in the United States, the Food and Drug Administration has classified this drug as a Schedule I controlled substance, having a high potential for abuse with little accepted medical use. In order to study the actions of MDMA on the central nervous system we have synthesized a radiolabeled form of MDMA for use in uptake and binding studies on the rat brain.

Male Sprague-Dawley rats (200-250 g) were killed by decapitation and the whole brain excised. Brain tissue was homogenized in 50 mM Tris (pH 7.4) using a Brinkman Polytron on setting 6 and centrifuged at $45\,000 \times g$ for 10 minutes at 4°C. This was repeated twice. Binding studies were performed in a modified Krebs phosphate buffer (pH 7.7). [^{3}H](±)-MDMA HCl was synthesized by the reaction of (3,4-methylenedioxyphenyl)-2-propanone (Fluka AG, Switzerland) with [^{3}H]methylamine (SA 30 Ci/mmol, Amersham, Arlington Hts., IL) in the presence of sodium cyanoborohydride with subsequent purification by thin layer chromatography. Unlabeled MDMA HCl was synthesized by an identical procedure and had a purity in excess of 99.5% as determined by NRM and elemental analysis.

Initial studies indicated that [^{3}H](±)-MDMA binding rapidly reach equilibrium at 4°C (optimal temperature) and was readily reversible upon addition of unlabeled MDMA. Equilibrium binding studies were conducted using ligand concentrations of 1-300 nM and a final protein concentration of 1-2 mg/ml. Incubations were terminated by rapid filtration using a Brandel (Gaithersburg, MD) Cell Harvester and Whatman GF/C filters. The filters were washed three times (3 ml) with ice cold 50 mM Tris buffer (pH 7.7), placed in scintillation vials and the radioactivity bound to the tissue assessed using liquid scintillation counting techniques. Nonspecific binding was defined as the radioactivity remaining after coincubation with 50 μM unlabeled MDMA.

Specific binding of [^{3}H](±)-MDMA constituted approximately 50–60% of the binding in the concentration dependent range of the saturation curve. Nonspecific binding was substantially lowered by using GF/C filters rather than GF/B. Pretreatment of the filters with 0.1% polyethylenimine did not result in reduced nonspecific binding. Scatchard analysis of [^{3}H](±)-MDMA binding gave an apparent dissociation constant of 99 nM and a B_{max} of 30 fmol/mg protein (figure 1). Hill plot analysis gave a slope of 1.01 indicating

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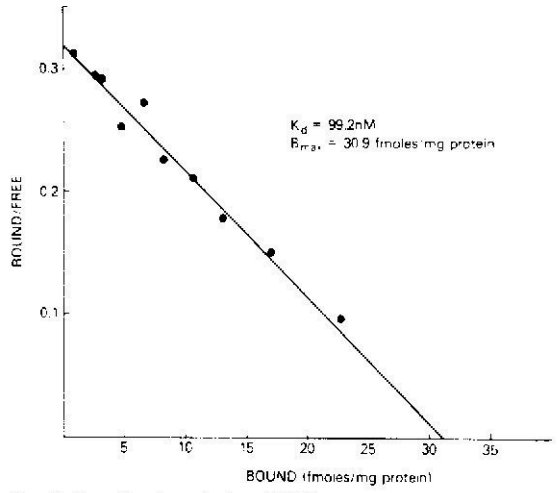

Fig. 1. Scatchard analysis of [^{3}H](±)-MDMA binding at 4°C. Rat whole brain membranes were incubated with concentrations of [^{3}H](±)-MDMA HCl ranging from 1 to 300 nM as described in the text. The data shown are from a representative experiment.

little cooperativity was involved in the binding of [^{3}H](±)-MDMA. The specifically bound radioactivity could be inhibited by an approximately equimolar concentration of (±)-MDMA. Significant inhibition of binding was also observed with (±)-p-chloroamphetamine and (±)-methamphetamine but not d-amphetamine. Little displacement was observed when samples were incubated with ketanserin, methysergide, serotonin, fluorophenylethylamine, spiperone, propranolol, tyramine, pargyline, cyproheptadine, citalopram or atropine.

Our results indicate specific binding sites for MDMA exist in the rat brain. Studies performed in our laboratory have indicated that (±)-MDMA is a potent releaser of serotonin in vitro (Schmidt and Lovenberg, unpublished observations) while in vivo, a single dose of MDMA reduces striatal serotonin levels for up to 7 days after a single dose (Schmidt et al., unpublished observations). This latter effect is indicative of serotonergic neurotoxicity and can be blocked by coadministration of citalopram, a serotonergic reuptake inhibitor. Interestingly, citalopram did not antagonize the binding of [^{3}H](±)-MDMA in the current study and in vitro uptake studies performed using striatal synaptosomes indicate that [^{3}H](±)-MDMA is probably not concentrated in nerve terminals (data not shown). The results suggest MDMA may possess a number of pharmacological activities in the mammalian CNS. We are initiating studies with the labeled and unlabeled optical isomers of MDMA to determine which isomers are active as a neurotoxic or psychotropic agent.

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References

Anderson, G.M., G. Braun, U. Braun, D.E. Nichols and A.T. Shulgin, 1978, Absolute configuration and psychotomimetic activity, in: Quasar Research Monographs, eds. G. Barnett, M. Trsic and R. Willette (NIDA) p. 8.

Dowling, C.G., E. Barnes, S. Peters and J. Zich, 1985, The trouble with ecstasy, Life 8(9), 88.

Shulgin, A.T. and D.E. Nichols, 1978, Characterization of three new psycotomimetics, in: The Psychopharmacology of Hallucinogens, eds. R.C. Stillman and R.E. Willette (Pergamon Press, New York) p. 74.