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[^{3}H]3,4-Methylenedioxymethamphetamine (MDMA) interactions with brain membranes and glass fiber filter paper

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The binding of [^{3}H]3,4-methylenedioxymethamphetamine ([^{3}H]MDMA) to both rat brain membrane preparations and glass fiber filter papers was analyzed in the present study. Saturation studies indicate that [^{3}H]MDMA binding is saturable and monophasic in both the presence and absence of rat brain membranes. This apparent 'specific' binding of [^{3}H]MDMA in both the presence and absence of brain homogenates was totally eliminated by pretreating glass fiber filter papers with polyethylenimine. Drug competition studies demonstrated that [^{3}H]MDMA binding displays a distinct 'pharmacological' profile in the absence of brain tissue. These data indicate that apparent [^{3}H]MDMA binding to rat brain homogenates results from artifactual of [^{3}H]MDMA to glass fiber filter paper. In addition, uptake of [^{3}H]MDMA into rat brain synaptosomes could not be detected. We conclude the [^{3}H]MDMA has limited usefulness in radioligand binding studies.

3,4-Methylenedioxymethamphetamine (MDMA); Radioligand binding studies; Polyethylenimine; (Artifactual binding)

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is a psychotropic drug which possesses mild stimulant properties in man (Shulgin et al., 1969). The drug is chemically related to both stimulants such as amphetamine and hallucinogens as mescaline. MDMA is also a popular illicit drug at the present time. Like its structural analogue 3,4-methylenedioxyamphetamine (MDA), MDMA is a selective serotonergic neurotoxin (Ricaurte et al., 1985; Stone et al., 1986; Schmidt et al., 1986). The neurochemical basis of the psychoactive and neurotoxic effects of MDMA remains unknown.

Radioligand binding techniques have greatly facilitated the analysis of drug action in the central nervous system. A preliminary report by Gehlert et al. (1985) indicated that [^{3}H]MDMA appeared to label 'specific binding sites' in rat brain. The present study attempted to further characterize the binding of [^{3}H]MDMA to both rat brain membrane preparations and glass fiber filter papers and to assess its ability to be sequestered into rat brain synaptosomes.

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2. Materials and methods

2.1. Radioligand studies

Receptor binding assays were performed according to the methods of Gehlert et al. (1985). In brief, adult rat brains were purchased from Pel-Freeze, Inc. (Rogers, AR) and stored at −70 °C. On the day of the study, the brains were defrosted and cerebella were discarded. Remaining tissues were homogenized in 20 volumes of 50 mM Tris-HCl (pH 7.7 at 25 °C) using a Brinkmann Polytron and then centrifuged in an IEC

B20A centrifuge at 45000 × g for 10 min. The supernatant was discarded and the pellet was resuspended in 20 volumes of a Krebs original phosphate buffer (mM: 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4 \cdot 7H_2O$, 16 $Na_2HPO_4 \cdot 2H_2O$ and 3.2 HCl) pH 7.4. The homogenates were immediately used in the binding assay.

Saturation studies consisted of 25 µl of a solution of [^{3}H]MDMA (final concentrations = 4-512 nM), 25 µl of buffer or displacing drug and 200 µl of tissue suspension or buffer. All other radioligand studies were performed in a 1 ml final volume. Following incubation at 25° C for 30 min, the assay mixtures were rapidly filtered using a Brandel Cell Harvester (Gaithersburg, MD) and No. 32 glass fiber filters (Schleicher and Schuell, Keene, NH). The filters were washed two times with 3 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.7). Radioactivity was measured by liquid scintillation spectroscopy in 5 ml of 3a70 Counting Cocktail (Research Products International, Mount Prospect, IL) at 55% efficiency. Specific binding was defined as the excess over blanks taken in the presence of 10^{-4} M MDMA.

In additional studies, radioligand studies were performed as described above, with the exception that glass fiber filters were pre-soaked for 3 h in fresh 0.3% polyethylenimine (PEI), pH 10 (1 : 33 dilution of 10% stock PEI in distilled water).

2.2. Uptake studies

Analysis of [^{3}H]MDMA and [^{3}H]5-hydroxytryptamine (5-HT) uptake by crude cortical synaptosomal suspensions was performed as described previously (Ricaurte et al., 1980). Rat cortical tissues were homogenized in 9 volumes (weight per volume) of 0.32 M sucrose and centrifuged at 1000 × g for 10 min. The supernatant was decanted and used as a crude synaptosomal suspension. Of this suspension 0.1 ml was added over ice to 1.9 ml of a Krebs phosphate buffer (pH 7.4) which contained either [^{3}H]5-HT or [^{3}H]MDMA at final concentrations of 25-500 nM. After vortexing, all tubes (except 0-4° C blanks) were incubated at 37° C for 5 min and then returned to ice. Tube contents were then rapidly filtered using a Brandel Cell Harvester with No. 32 glass fiber filters. Filters were washed three times with 3 ml of ice-cold 0.9% NaCl, then measured for radioactivity by liquid scintillation spectroscopy. Active uptake was defined as the difference in ^{3}H accumulation at 37 and 0-4° C.

2.3. Drugs

All drugs were dissolved and diluted in Krebs buffer. Drug sources were as follows: [^{3}H]MDMA (74.7 Ci/mmol) was generously provided by Dr. Steven Hurt (Dupont-New England Nuclear, Boston, MA). MDMA and 3,4-methylenedioxyethamphetamine (MDE) were generously provided by Dr. David Nichols of Purdue University. Other drugs were obtained from the following sources: 4-iodo-1-(2,5-dimethoxyphenyl-2-aminopropane (DOI), 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT) and (+)-butaclamol (Research Biochemicals, Inc., Waltham, MA); 5-HT, diazepam and amitriptyline (Sigma Chemical Co., St. Louis, MO); d-lysergic acid diethylamide (d-LSD) and MDA (National Institute on Drug Abuse, Bethesda, MD); cinanserin (E.R. Squibb and Sons, Inc., Princeton, NJ); fluoxetine (Lilly Research Laboratories, IN); and mesulergine (Sandoz, East Hanover, NJ).

3. Results

3.1. Saturation analysis of [^{3}H]MDMA binding to rat brain membranes and glass fiber filter paper

Increasing concentrations of [^{3}H]MDMA were incubated in the presence or absence of rat brain homogenates. In the presence of rat brain membranes, the specific binding of [^{3}H]MDMA is saturable. Scatchard analysis is monophasic, giving an averaged K_D value of 92 ± 20 nM and a B_{max} of 5.54 ± 1.0 pmol/g tissue (n = 3). A representative Scatchard analysis is shown in fig. 1A. The same experiment was performed in the absence of rat brain homogenates. Increasing concentrations of [^{3}H]MDMA were incubated in a final volume of 250 µl of assay buffer. Scatchard

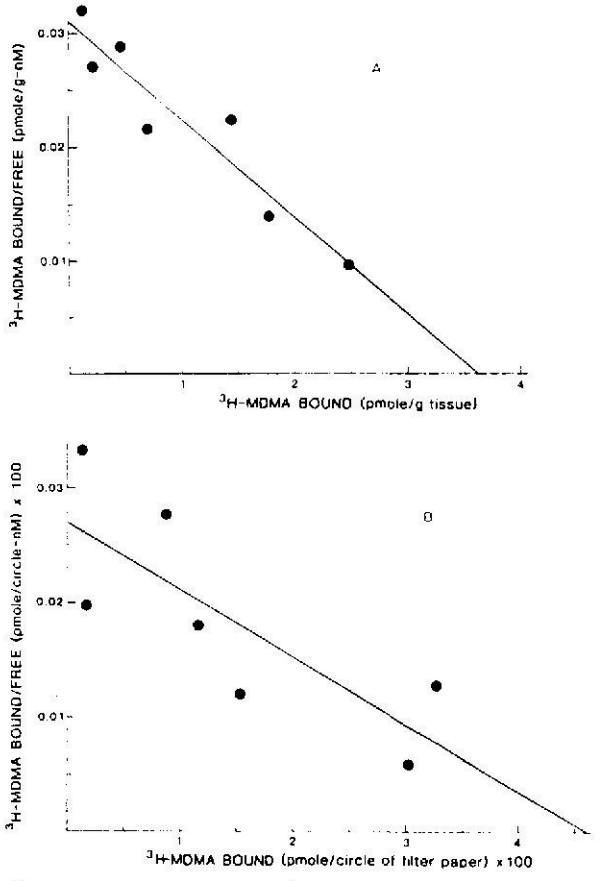

Fig. 1. Scatchard analysis of [^{3}H]MDMA binding in the presence and absence of rat brain homogenates. Saturation experiments were performed using increasing concentrations of [^{3}H]MDMA (4-512 nM) in the presence (A) or absence (B) of rat brain homogenates. Binding assays were performed as described in Materials and methods. 'Specific' binding was defined as the excess over blanks taken in the presence of 10^{-4} M MDMA. Data are the means of triplicate assays performed in a single experiment. Each experiment was repeated three times with similar results. Linear regression was used to determine the axis intercept points. (A) Rat brain: $K_d = 120$ nM; $B_{max} = 3.6$ pmol/g tissue or 0.036 pmol/circle of filter paper. (B) No tissue: 'K_d' = 170 nM; 'B_{max}' = 0.046 pmol/circle of filter paper.

analysis in the absence of tissue is also monophasic (correlation coefficient = 0.88; fig. 1B). Despite the absence of tissue, Scatchard analysis of [^{3}H]MDMA binding to glass fiber filters has a K_D value of 150 ± 20 nM and a B_{max} value of 0.057 ± 0.011 pmol/circle of filter paper ($n = 3$). This value for B_{max} is not statistically different from the value obtained per filter paper that is observed in the presence of rat brain homogenates (i.e. 0.055 pmol/circle of filter paper).

3.2. Effect of filter pre-treatment with PEI on [^{3}H]MDMA binding

In the presence of rat brain homogenates, total binding of 8.4 nM [^{3}H]MDMA represented 2000 $\pm$ 50 d.p.m., whereas 'non-specific' binding represented 1420 ± 70 d.p.m. In the absence of tissue, total [^{3}H]MDMA binding represented 2220 ± 10 d.p.m. whereas non-specific binding represented 1180 ± 50 d.p.m. Thus, apparent specific binding accounted for 29% specific binding in the presence of tissue and 47% specific binding in the absence of tissue.

When radioligand studies using rat brain homogenates were performed using glass fiber filters pretreated with 0.3% PEI, total [^{3}H]MDMA binding represented 1600 ± 40 d.p.m. while non-specific binding was 1640 ± 40 d.p.m. Interestingly, in assays without tissue, both total and non-specific [^{3}H]MDMA binding decreased significantly to 550 ± 20 d.p.m. and 550 ± 20 d.p.m., respectively. Thus, the apparent specific binding of [^{3}H]MDMA was eliminated in both the presence and absence of brain homogenates by filter pretreatment with PEI.

3.3. Drug interactions with specific [^{3}H]MDMA binding to glass fiber filter paper

The ability of drugs to compete for specific [^{3}H]MDMA binding to glass fiber filter paper was determined in the absence of brain homogenates. As shown in table 1, a number of pharmacological agents significantly decreased the specific binding of [^{3}H]MDMA to glass fiber filter paper. The three agents which were most potent in inhibiting specific [^{3}H]MDMA binding were MDMA, MDA and MDE. These agents are ring-substituted amphetamines and are structural analogs. The next most potents were fluoxetine and amitriptyline. While structurally diverse, these agents are similar in that they are both potent inhibitors of serotonergic uptake systems. By contrast, serotonergic drugs such as 8-OH-DPAT, DOI,

TABLE 1

Drug competition with 'specific' [^{3}H]MDMA binding to glass fiber filter paper. Radioligand studies were performed as described in Materials and methods using 4 nM [^{3}H]MDMA. Competition studies were performed in the presence of 10^{-7} M unlabeled drug (n = 3). Specific binding was defined as the excess over blanks taken in the presence of 10^{-4} M MDMA.

10^{-7} M competing drug	% of total [^{3}H]MDMA binding displaced
MDMA	56 ± 5
MDA	43 ± 5
MDE	41 ± 4
Fluoxetine	36 ± 2
Amitriptyline	31 ± 8
8-OH-DPAT	29 ± 5
DOI	29 ± 10
(+)-Butaclamol	24 ± 7
d-LSD	24 ± 3
Diazepam	21 ± 6
5-HT	21 ± 10
Mesulergine	2 ± 7
Cinanserin	1 ± 10

d-LSD and 5-HT show only moderate ability to compete for a specific [^{3}H]MDMA binding to glass fiber filter paper. Finally, both cinanserin and mesulergine, which are potent 5-HT$_2$ receptor antagonists, are essentially inactive in competing for specific [^{3}H]MDMA binding.

3.4. Uptake studies of [^{3}H]MDMA and [^{3}H]5-HT using synaptosomes

Increasing concentrations of [^{3}H]MDMA and [^{3}H]5-HT were incubated in the presence of crude cortical synaptosomal suspensions. No uptake of [^{3}H]MDMA was evident in concentrations ranging from 25-500 nM. By contrast, uptake of [^{3}H]5-HT under identical conditions was saturable and sensitive to fluoxetine inhibition. The apparent K_m and V_{max} for [^{3}H]5-HT uptake were 0.10 ± 0.02 μM and 24 980 ± 1 780 d.p.m., respectively.

4. Discussion

The major finding of the present study is that apparent specific [^{3}H]MDMA binding to rat brain homogenates results from labeling of glass fiber filter papers. This apparent, but artifactual, binding of the radioligand is saturable and monophasic. Drug competition studies reveal a specific 'pharmacological profile' of drug interactions with the [^{3}H]MDMA binding to glass fiber filter paper. Thus, ring-substituted amphetamines and serotonergic uptake inhibitors are the most potent agents in competing for this specific but artifactual binding site. Pretreatment of glass fiber filters with PEI, an agent which has been reported to reduce non-specific interactions between radioligands and glass fiber filter papers (Bruns et al., 1983), totally eliminates apparent specific binding of [^{3}H]MDMA in both the presence and absence of brain homogenates.

Radioligand binding studies have been extremely useful in characterizing the site of action of pharmacological agents in the central nervous system. Although a variety of criteria have been established to confirm that radioligand 'binding sites' are equivalent to membrane receptors, these criteria are frequently overlooked in initial reports of novel radioligands. For example, radioligand binding to glass tubing and other inorganic materials can cause significant problems with interpretation of radioligand data (Yamamura et al., 1978). Radioligands such as [^{3}H]8-OH-DPAT (Peroutka and Demopulos, 1986) have been found to label glass fiber filter paper under certain conditions.

The present study is an excellent example of such problems. For example, the binding of [^{3}H]MDMA to glass fiber filter paper has a very unique and interesting pharmacological profile. The most potent agents are structural analogues, i.e. ring-substituted amphetamine derivatives. The next most potent agents are both serotonin uptake inhibitors. Since fluoxetine and amitriptyline are structurally diverse, a possible interpretation of the present data could be that [^{3}H]MDMA labels part of the serotonergic uptake system. In fact, the ability of [^{3}H]MDMA to 'specifically' label glass fiber filter papers suggests that the ionic properties of the filter paper allows [^{3}H]MDMA to compete for specific but physiologically irrelevant sites on the filter paper. Therefore, [^{3}H]MDMA cannot be used to analyze potential brain membrane binding sites unless its binding to glass fiber filter paper is eliminated.

The second phase of this study attempted to analyze the ability of [^{3}H]MDMA to be sequestered in rat brain synaptosomes. Under identical conditions, the uptake of [^{3}H]5-HT is rapid and saturable. By contrast, there was no evidence that [^{3}H]MDMA could be sequestered into rat brain synaptosomes at concentrations between 25 and 500 nM. Despite the fact that ring-substituted amphetamines destroy 5-HT terminals, these data suggest that the mechanism of action does not include direct uptake of MDMA into synaptosomes.

Therefore, the present study demonstrates that [^{3}H]MDMA labels glass fiber filter papers and cannot be sequestered into rat brain synaptosomes. Thus, despite a preliminary study suggesting that [^{3}H]MDMA labels a specific brain recognition site, we conclude that [^{3}H]MDMA has extremely limited usefulness in characterizing the potential site or sites of action of MDMA.

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References

Bruns, R.F., K. Lawson-Wendling and T.A. Pugsley, 1983, A rapid filtration assay for soluble receptors using polyethylenimine-treated filters, Anal. Biochem. 132, 74.

Gehlert, D.R., C.J. Schmidt, L. Wu and W. Lovenberg, 1985, Evidence for specific methylenedioxymethamphetamine (Ecstasy) binding sites in the rat brain, European J. Pharmacol. 119, 135.

Peroutka, S.J. and C.M. Demopulos, 1986, [^{3}H]8-OH-DPAT 'specifically' labels glass fiber filter paper, European J. Pharmacol. 129, 199.

Ricaurte, G., G. Bryan, L. Strauss, L. Seiden and C. Schuster, 1985, Hallucinogenic amphetamine selectively destroys brains serotonin nerve terminals, Science 229, 986.

Ricaurte, G.A., C.R. Schuster and L.S. Seiden, 1980, Long-term effects of repeated methamphetamine administration on dopamine and serotonin neurons in the rat brain, Brain Res. 193, 153.

Schmidt, C.J., L. Wu and W. Lovenberg, 1986, Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue, European J. Pharmacol. 124, 175.

Shulgin, A.T., T. Sargent and C. Naranjo, 1969, Structure-activity relationships of one-ring psychotomimetics, Nature 221, 537.

Stone, D.M., D.C. Stahl, G.R. Hanson and J.W. Gibb, 1986, The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain, European J. Pharmacol. 128, 41.

Yamamura, H.I., S.J. Enna and M.J. Kuhar, eds., 1978, Neurotransmitter Receptor Binding (Raven Press, New York).