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BRIEF COMMUNICATION

Methysergide Potentiates the Hyperactivity Produced by MDMA in Rats[1]

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GOLD, L H AND G F KOOB *Methysergide potentiates the hyperactivity produced by MDMA in rats* PHARMACOL BIOCHEM BEHAV **29**(3) 645–648, 1988 —Although some substituted amphetamines, like MDA, produce a combination of sympathomimetic stimulation and perceptual alterations, the psychoactive qualities of MDMA are less distinctive MDMA binds to serotonergic receptors and has been shown to potently deplete brain serotonin concentrations Biochemical and behavioral evidence suggests that MDMA may also act on the dopamine system The present study explored the effects of blocking serotonin receptors on MDMA and amphetamine induced locomotor hyperactivity in rats Locomotor activity was measured in photocell cages for 120 minutes following injection of methysergide (0, 2 5, 5, 10 mg/kg) or methysergide in combination with amphetamine (0 5 mg/kg) or MDMA (10 mg/kg) Methysergide, which had no effect on its own, significantly potentiated the locomotor hyperactivity produced by MDMA but not amphetamine Thus, the intrinsic serotonergic agonist properties of MDMA may actually counteract the indirect sympathomimetic effects thought to be responsible for the locomotor hyperactivity MDMA produces

Methylenedioxymethamphetamine MDMA Methysergide Locomotor activity

THERE has recently emerged a new category of recreational drugs called "designer drugs " This title refers to chemicals that are prepared to produce desirable physical effects [16] Amphetamine-like designer drugs (methylenedioxyamphetamine, MDA and methylenedioxymethamphetamine, MDMA) combine hallucinogenic activity with the classical stimulant actions of amphetamine. Variations in the location and identity of substituent groups can profoundly alter the ability of these compounds to elicit stimulant or psychotomimetic effects [25]. Thus, N-methylation of MDA to produce MDMA emphasizes the stimulant properties in preference to the psychedelic properties [24]

Such structural manipulations also confer differential neurochemical actions Subacute treatment of MDMA in rats causes a decrease in tryptophan hydroxylase, serotonin (5-HT) and the serotonin metabolite, 5-HIAA, measured in neostriatum, hippocampus and cortex [25] In contrast, repeated dosings of MDMA result in elevated homovanillic acid concentrations but do not alter striatal tyrosine hydroxylase activities or reduce striatal dopamine concentrations In vitro, MDMA potently releases [^{3}H]5-HT from preloaded rat striatal slices but is less effective at increasing [^{3}H]-dopamine release [23] In this same report, [^{3}H]5-HT uptake by a synaptosomal preparation was found to be significantly reduced one week following a single injection of MDMA These studies suggest that although MDMA produces alterations in dopaminergic systems, the long term effects of MDMA (activity attributed to the + isomer) may be due to neurotoxic effects on serotonergic neurons

In addition to its indirect releasing properties, studies of MDMA binding have found nearly equal affinity for 5-HT$_1$ and 5-HT$_2$ sites and low affinity for dopamine$_2$ sites [15]. A separate report described specific binding sites for MDMA in rat brain at which inhibition by PCA and methamphetamine was seen but little displacement was observed when the samples were incubated with serotonin, d-amphetamine, or various other aminergic agents [7] The fact that (−)R-MDMA was found to possess three fold greater serotonin binding affinity than the (+)-S enantiomer [15] contrasts with one report that the (+) enantiomer is more potent in human subjects [2] If this discrepancy is real, it suggests that the psychoactive effects of MDMA in man may be mediated by mechanisms other than direct serotonergic activation. Evidence is accumulating which demonstrates that multiple components of the action of hallucinogenic phenylethylamines may be responsible for effects ranging from LSD-like to amphetamine-like [15,18]

Clinically MDMA has been used as an adjunct to psychotherapy Psychiatrists report it enhances emotional sensitivity and awareness and increases effective communication [1, 11, 16] In contrast to MDA, MDMA is virtually devoid of hallucinogenic activity and has relatively mild

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sympathomimetic side effects [24] Due to the structural similarity of MDMA with other hallucinogens and amphetamine, and as a result of reports that MDA causes selective serotonin nerve terminal degradation [19] MDMA was assigned emergency Schedule I status in June, 1985 The purpose of the present study was to assess the functional similarities of MDMA and AMPH induced hyperactivity In particular, the role of serotonin in the stimulant actions of MDMA and AMPH was examined in rats who received the serotonin antagonist methysergide [4], concurrently with these drugs

METHOD

The subjects were eighty male, albino Wistar rats (220–320 g, Charles River, Kingston) housed in groups of three in a temperature controlled environment under a normal 12 hour light cycle (lights on 0700, lights off 1900) with free access to food and water Before behavioral testing, each rat was briefly handled by the experimenter (5 minutes) The study was conducted by performing three separate experiments

Locomotor activity was measured in a bank of 16 wire cages 20×25×36 cm each with two horizontal infrared beams across the long axis 2 cm above the floor Total photocell beam interruptions and crossovers were recorded by a computer every ten minutes

Before the drug series, each rat was habituated to the photocell cages overnight, and prior to drug injection the rats were habituated again to the photocell cages for at least 90 minutes Following drug administration, activity was measured for 120 minutes d-Amphetamine sulfate, (±)MDMA hydrochloride (provided by the National Institute on Drug Abuse) and methysergide maleate were dissolved in saline and injected SC in a volume of 1 ml/kg body weight In Experiment 1, all rats were injected with methysergide (0, 2 5, 5, 10 mg/kg) and then two minutes later with MDMA 10 mg/kg, N=8 rats/group In Experiment 2, all rats were injected with methysergide (0, 2 5, 5, 10 mg/kg) and then two minutes later with d-amphetamine at a dose of 0 5 mg/kg, N=6 rats/group (except AMPH/methysergide 5 0 mg/kg group, N=5 due to equipment problem) In Experiment 3, all rats were injected only with methysergide (0, 2.5, 5, 10 mg/kg), N= 6 rats/group Drug doses for amphetamine and MDMA were selected to produce similar increases in activity, although MDMA appears to have a longer duration of action (unpublished results, Gold, Koob and Geyer) Ten minute totals for locomotor activity were subjected to a two way analysis of variance (ANOVA) with repeated measures on the second factor, time Individual means comparisons for the main drug effects were analyzed using a Newman-Keuls *a posteriori* test

RESULTS

The locomotor activating properties of MDMA, amphetamine and methysergide are seen in Fig. 1 Once the rats were habituated to the photocell apparatus, saline injection produced only transient arousal (lasting less than 20 minutes) followed by relative inactivity (see Fig 1C) MDMA 10 mg/kg produced an increase in beam interruptions which lasted for at least two hours (Fig 1A) Two way ANOVA with repeated measures on time followed by Newman-Keuls individual means analyses revealed that methysergide (2 5, 5, 10 mg/kg) significantly potentiated the locomotor hyperactivity produced by MDMA 10 mg/kg when compared

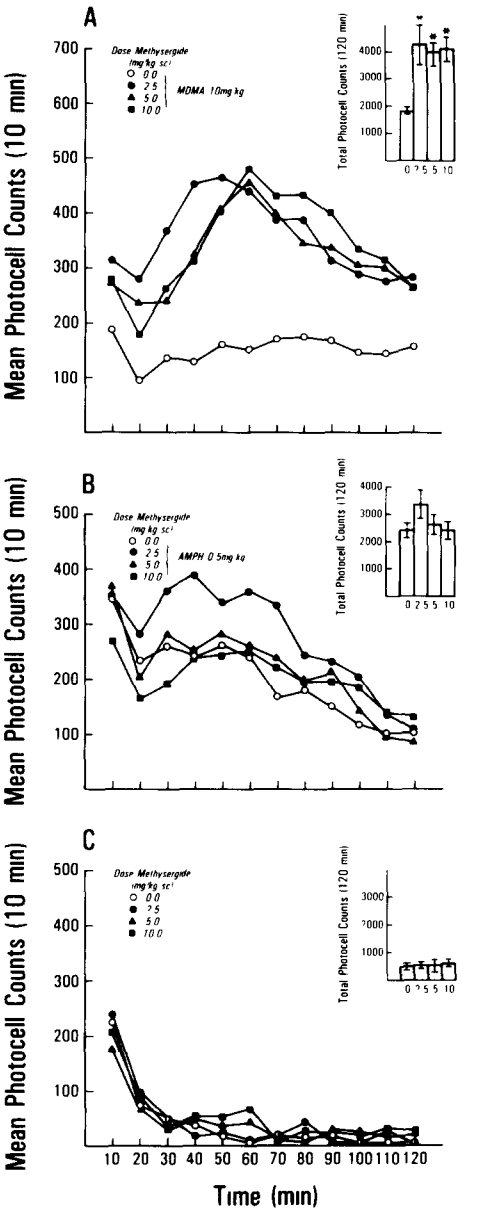

FIG 1 Locomotor activity during 120 minute test session Following a habituation period rats were injected with methysergide (0–10 mg/kg SC, C) and 2 minutes later by (A) MDMA (10 mg/kg SC), (B) amphetamine (0 5 mg/kg SC) Values in the upper right corner of each panel represent mean±SEM for the total activity over the 2 hr drug test *Significantly different from 0 methysergide dose, Newman-Keuls test following significant ANOVA main effect

to MDMA injection alone. [Main effect $F(3,28)=5.59$, dose × time interaction: $F(33,308)=3\ 16$, $p<0.05$, Fig. 1A] This enhancement of MDMA's locomotor effects was evident within the first ten minutes measured and lasted for the full two hour session In contrast, methysergide only slightly increased the locomotor hyperactivity produced by 0 5 mg/kg of amphetamine (Fig 1B) This effect was not statistically significant [main effect $F(3,19)=1\ 39$, dose × time interaction $F(33,209)=1.24$, $p>0.05$] Methysergide alone had no effect on locomotor activity [Fig 1C, main effect

$F(3,20)=<1.0$, dose × time interaction $F(33,220)=1.08$, $p>0.05$] when compared to saline injection. The effects on crossovers were not qualitatively different from beam interruptions and therefore are not reported.

DISCUSSION

The anatomical organization of monoaminergic systems as well as biochemical and pharmacological data support a role for both catecholamines and serotonin in controlling some aspects of motor behavior in rats [21]. Furthermore, it has been suggested that serotonergic inhibition modulates catecholamine-mediated arousal. In general, manipulations which decrease brain serotonin have been found to increase responses to catecholamine agonists such as amphetamine and enhancement of serotonin activity is associated with reduced responses [8]. Marby and Campbell [17] observed a potentiation of amphetamine induced locomotor activity in rats by the serotonin biosynthesis inhibitor PCPA and suppression of this effect by the serotonin precursor 5-hydroxytryptamine. Similarly, interruption of the serotonergic fibers in the medial forebrain bundle and depletion of serotonin produced an enhancement of amphetamine action as measured by increased rates of responding on a schedule of reinforcement [10]. Indeed, it has been suggested that forebrain serotonin and catecholamine neurons exert reciprocal effects on various behaviors [9].

The present study demonstrates that the stimulant properties of MDMA are enhanced by the presence of a serotonin antagonist, methysergide. Thus, following serotonin receptor blockade, profound locomotor hyperactivity was observed. These data are consistent with the hypothesis that MDMA acts predominantly as a serotonin agonist with weaker dopamine activity [25]. The serotonin agonist properties intrinsic to MDMA may explain the somewhat blunted locomotor activation compared to amphetamine seen in rats (this study) and subjective reports of more mild sympathetic arousal in man [11,24].

In the present experiment, methysergide did not potentiate the effect of amphetamine. While there is a small difference in the amount of locomotor activity produced by MDMA versus amphetamine, it is always difficult to select doses of drugs that will produce identical behavioral effects. In fact, the dose of amphetamine chosen is not maximal [6] so we believe the results are not due to a ceiling effect. However, Hollister *et al* [12] reported that methysergide potentiated locomotion produced by 2 mg/kg amphetamine (IP). The higher dose of amphetamine and different route of administration in that study may explain this difference. Similar inconsistencies also exist with regard to the effects of serotonin antagonism on stereotyped behavior produced by dopamine agonists. Weiner *et al* [26] reported that methysergide enhanced both amphetamine- and apomorphine-induced stereotypy, whereas, Rotrosen *et al* [20] observed no effect of methysergide on apomorphine induced stereotypy in rats. These results plus those of the present study suggest that the exact relationship between serotonin and catecholamines in behavioral arousal produced by indirect sympathomimetics may require more systematic study using other substituted amphetamines.

The pharmacology of MDMA in animals is currently being investigated in other behavioral paradigms. Drug discrimination studies in pigeons [5] and monkeys [13] have shown that MDMA produces drug appropriate responding in animals trained to discriminate amphetamine from saline. Drugs which share discriminative stimulus properties are thought to have at least some subjective effects in common. In rats, MDMA generalizes to fenfluramine and tetrahydro-β-carboline (THBC) as well as l-cathinone [22]. This duality of effects was interpreted to suggest that MDMA may be acting both as an indirect dopamine agonist and as a serotonergic receptor agonist, a conclusion consistent with the present results. In addition, potential for abuse has been demonstrated in animal models of self administration. MDMA maintained more injections and higher response rates than were maintained by saline in Rhesus monkeys [3] and baboons [14] trained to self administer cocaine.

A common underlying mechanism for stimulant action may contribute to the stimulus generalization of MDMA to amphetamine and cocaine and its self administration seen in preclinical behavioral tests. Further neuropharmacological characterization is needed before MDMA is classified as a psychedelic, hallucinogen or simply a stimulant. To the extent that psychomotor activity is an important aspect of the reinforcing qualities of drugs, then MDMA, like other classic stimulants, would seem to have similar potential for abuse. However, results from the present study demonstrate that the intrinsic serotonergic agonist activity of MDMA may partially inhibit this effect.

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