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Reinforcing Effects of Certain Serotonin-Releasing Amphetamine Derivatives

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MARONA-LEWICKA, D., G.-S. RHEE, J. E. SPRAGUE AND D. E. NICHOLS. *Reinforcing effects of certain serotonin-releasing amphetamine derivatives.* PHARMACOL BIOCHEM BEHAV 53(1) 99–105, 1996. – The present study was designed to characterize further the rewarding and aversive properties of 3,4-methylenedioxymethamphetamine (MDMA), the α-ethyl homologue of MDMA (MBDB), fenfluramine, and the selective serotonin releasing agent 5-methoxy-6-methyl-2-aminoindan (MMAI) using the conditioned place preference paradigm (CPP). Extracellular dopamine (DA) and its metabolite DOPAC were also measured in the nucleus accumbens after systemic drug administration, using in vivo microdialysis in freely moving rats. MDMA produced a positive dose-dependent effect in the CPP test, which was maximal at doses of 5 and 10 mg/kg. MBDB also induced a positive CPP, with a maximum effect at 10 mg/kg. The conditioning effect of MBDB was more than 2.5-fold weaker compared with MDMA. Fenfluramine evoked place aversion at doses of 4, 6, and 10 mg/kg. This effect of fenfluramine was independent of dose. MMAI at doses of 1.25, 2.5, and 5 mg/kg produced no significant effect on place conditioning. At doses of 10 and 20 mg/kg, MMAI produced an effect similar to fenfluramine: Place aversion was independent of dose. In the microdialysis experiments, MDMA significantly elevated extracellular DA and induced a decrease of DOPAC in the nucleus accumbens. Thus, activation of dopaminergic systems may be responsible for the rewarding properties of MDMA-like drugs. In contrast to the effects seen with MDMA, no difference in extracellular DA or DOPAC was seen after injection of MBDB, fenfluramine, or MMAI, even though MBDB weakly induced a place preference. The mechanism responsible for the development of place aversion by fenfluramine or MMAI is unknown at this time and requires further study.

Conditioned place preference MDMA MBDB Fenfluramine MMAI Reward Place aversion
In vivo microdialysis in freely moving rats Dopamine Nucleus accumbens

THE REINFORCING efficacy of drugs is typically tested in behavioral paradigms that include drug self-administration, facilitation of intracranial electrical self-stimulation, and place conditioning. The conditioned place preference (CPP) paradigm is a relatively simple model that has been used to assess the affective properties of various drugs, including stimulants and opiates (14). This procedure is based on the fact that the pairing of distinctive but neutral environmental stimuli with a primary reward (a drug) will result subsequently in an acquired preference for those specific stimuli in the absence of the primary reward. Because drugs that have reinforcing properties are also considered to have a higher probability of dependence liability and substance abuse, the ability of a compound to produce a place preference may be predictive of such properties. The advantage of using the place-conditioning paradigm is its sensitivity not only to reinforcing properties but also the aversive properties of drugs. A place preference is defined as a greater preference for a drug-paired chamber than the saline-paired chamber. A place aversion is defined as a greater avoidance of the drug-paired chamber than the saline-paired chamber (80).

Our interest in indirectly acting serotonergic agents was stimulated about 10 years ago by studies of the psychoactive phenethylamine derivative 3,4-methylenedioxymethamphetamine (MDMA) (2,55). This substance later became popular as a recreational drug, available on the street under the name "ecstasy" (62–64). The drug produces unique psychoactive effects, which differ from both classical psychostimulants and hallucinogenic agents (57). It is apparent that MDMA also produces a state that might be characterized as "rewarding"

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(33,64). That is, MDMA is self-administered by monkeys (5) and baboons (47). Furthermore, MDMA is able to produce CPP in rats, an index of drug-rewarding properties (6–8,69).

Studies in rodents and primates have also demonstrated that acute and/or repeated administration of MDMA produces long-term reductions in the brain concentration of 5-HT (serotonin) and its metabolite 5-HIAA (5-hydroxyindoleacetic acid) (4,18,73,78), as well as the destruction of central 5-HT nerve terminals (60,72).

Another amphetamine derivative, fenfluramine, has similar MDMA-like 5-HT releasing/uptake blocking properties (19,22,35,68). Fenfluramine is widely marketed in Europe as an agent to suppress appetite (50), even though considerable evidence from numerous laboratories indicates that fenfluramine produces long-lasting central serotonergic deficits (3,10, 37,43,51,81,84). All of these studies would seem to suggest certain similarities between the actions of MDMA and fenfluramine. Nevertheless, the human psychopharmacology of MDMA and fenfluramine is quite different. Whereas psychoactive properties of fenfluramine are not normally detectable at anorectic doses, MDMA used recreationally produces clear changes in affect somewhat similar to, but nevertheless distinct from, those produced by psychomotor stimulants (17, 64). From the point of view of development of a model for the central action of substituted amphetamines, this disparity in effects has been a troubling aspect of drug discrimination studies in our laboratory, where MDMA and fenfluramine appear to produce a similar or identical interoceptive cue in rats (57,59).

On the other hand, and in contrast to MDMA, fenfluramine does not appear to produce or maintain drug self-administration (1,26,29,65,80), or facilitate responding for electrical stimulation to the lateral hypothalamus (44). When assessed in these paradigms, fenfluramine does not serve as a reinforcing agent. Indeed, it has been demonstrated that fenfluramine effectively establishes a conditioned aversion to taste with which it is paired in a conditioned avoidance paradigm (9,23,27,28) and conditioned place aversion (21).

Over the past several years, we have identified in our laboratory a variety of structures that are potent releasers of neuronal serotonin, many of which lack serotonergic neurotoxic properties. One of these, 5-methoxy-6-methyl-2-aminoindan (MMAI), is a nonneurotoxic and highly selective serotonin releasing agent (39,49,56). It was reported from drug discrimination studies that symmetrical substitution occurs between MDMA, fenfluramine, and MMAI (49,59). From those data we concluded that all of these agents have a similar mechanism of action in which release of neuronal 5-HT has a primary role. However, other behavioral tests (e.g., locomotor activity, serotonin syndrome) suggested that MMAI-induced behavior was similar to that seen after fenfluramine administration, and different from that following MDMA (12,49). Although it is known that fenfluramine produces conditioned place aversion, the effect of MMAI on place conditioning has not been assessed.

The rewarding effects of amphetamine appear to depend critically on its actions in central dopamine (DA) pathways. Neurotoxin lesioning of DA terminals in the nucleus accumbens blocked amphetamine self-administration (48) and CPP (76). Carr and White (15,16) demonstrated that unilateral injections of DA or amphetamine into the nucleus accumbens produced a CPP. Although the basic pharmacology of MDMA differs considerably from amphetamine, both drugs potently increase synaptic transmission in catecholaminergic pathways (11,36,52,55,67). The neurochemical mechanisms underlying MDMA's reinforcing properties are unclear. However, it might be reasonable to suppose that the increased dopaminergic activity following MDMA administration may be critical for producing these effects (7,8).

For these reasons, we conducted a study to determine the relationship between the effects of MDMA, fenfluramine, and MMAI on place conditioning and changes in extracellular DA in rat nucleus accumbens following the administration of these drugs. To test the hypothesis that increased dopaminergic synaptic transmission is efficient for conferring rewarding properties to these drugs, we included the α-ethyl homologue of MDMA, MBDB [(±)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine]. Indeed, MBDB has been shown to have effects in humans similar to those of MDMA (75), and fully mimicked MDMA in a drug discrimination study (59). Numerous studies in our laboratory and others, however, have demonstrated that a major salient feature of MBDB that distinguishes it from MDMA is its relative lack of effect on dopaminergic systems, either in vivo or in vitro (40,41,53,77). We therefore investigated the rewarding and aversive properties of MDMA, MBDB, fenfluramine, and MMAI using the CPP paradigm, and examined the relationship between this effect and changes in extracellular DA and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), in rat nucleus accumbens using in vivo microdialysis in awake, freely moving rats.

METHODS

Animals

Male Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing 180–200 g at the beginning of the experiment, were used for the CPP paradigm and microdialysis studies. Animals were housed five per cage with free access to food (Lab Blox; Purina, Richmond, VA) and water, under a 12 L : 12 D cycle. None of the rats had previously received drugs or behavioral training. All animals were adapted to laboratory conditions for at least 1 week and were handled during this adaptation period for 10 min each day. Behavioral experiments were performed between 0900 and 1700 h.

CPP: Apparatus and Procedure

The apparatus for behavioral testing was a rectangular shuttle-box (Lafayette Instruments, Lafayette, IN) divided into two compartments of equal size (23 × 31 × 21 cm) by a guillotine door. One compartment had unpainted metal walls and a grid floor; the other had black-painted walls and a smooth metal floor.

On 4 consecutive days, the animals were placed in the center area (the door was raised), and each rat was allowed to explore the apparatus for 15 min. The time spent by the rat in each compartment during the session on the 4th day was recorded (preconditioning test). The compartment in which the rat spent less time was designated the nonpreferred side; the other was the preferred side.

Conditioning training lasted 8 days. On days 5, 7, 9, and 11, animals received injections of different doses of MDMA, MBDB, MMAI, fenfluramine, or saline (control group) 30 min before being placed into the nonpreferred compartment for 20 min. On alternate days 6, 8, 10, and 12, animals were injected with saline 30 min before being placed into the preferred compartment for 20 min. The door connecting the two chambers was closed during conditioning training.

On day 13, 24 h after the last conditioning training, each rat was allowed to explore the apparatus freely for 15 min

with the guillotine door opened (postconditioning test). On this test day, neither saline nor drug was injected. Time spent in each chamber was recorded as described for the preconditioning test.

Surgery, Microdialysis Procedure, and Histology

Microdialysis probes with a 2-mm-long dialysis membrane attached to a stainless-steel shaft were constructed as described by Yamamoto and Pehek (82). Recoveries for these probes were measured in the range of 15–17%. Rats were then anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg), and microdialysis probes were stereotaxically placed into the nucleus accumbens [A +3.6, L +1.8, V −7.0 from bregma; (61)]. The probes were then fixed in place with dental acrylic supported by a stainless-steel anchor screw threaded into the skull. The animals were given a 24-h recovery period before test procedures were begun.

After the recovery period, rats were placed into plastic cages. The dialysis probe was connected to a microinfusion pump (Carnegie Medicin, Stockholm, Sweden) calibrated to deliver Ringer's solution at a rate of 3.0 ml/min. The probe was perfused for at least 60 min to allow equilibration. Dialysate samples were then collected every 20 min. After the equilibration period, two baseline samples were collected, followed by a saline injection (T = 0 min). Three more samples were then collected at T = 20, 40, and 60 min before treatment with MDMA (6.3 mg/kg), MBDB (7.0 mg/kg), fenfluramine (7.7 mg/kg), or MMAI (6.2 mg/kg) (T = 60 min, immediately following the third sample collection). Five additional samples were collected after drug treatments. Doses selected were those found to be maximally effective in the CPP study.

After experiments, rats were decapitated. The brains were removed and postfixed first in 10% formalin for 24 h and then overnight in 30% sucrose solution. The brains were then sliced in 60-μm sections on a freezing microtome. The sections were mounted on microscope slides for verification of probe placement sites in the nucleus accumbens.

Biochemical Analysis

The concentrations of DA and DOPAC were determined in dialysate samples using high-performance liquid chromatography (HPLC) with electrochemical detection. A 50-μl aliquot from each sample was injected onto the HPLC column (Brownlee C18; Anspec, Ann Arbor, MI). The electrochemical detector was a model 400 EG&G Princeton electrochemical detector (Princeton, NJ) with series dual electrodes set at E_1 = −200 mV and E_2 = 850 mV vs. the Ag/AgCl reference electrode. This setup yielded a detection limit for dopamine of 0.8 pg/μl. The basal levels of DA in these studies ranged from 0.6–0.8 pg/μl and basal DOPAC levels ranged from 150–195 pg/μl. The mobile phase consisted of 50 mM NaH_2PO_4, 30 mM citric acid, 0.1 mM Na_2EDTA, 0.034% sodium octyl sulfate, and 25% methanol. Peaks were integrated with the Dynamax Methods Manager software (Rainin, Woburn, MA) implemented on an Apple Macintosh SE computer.

Drugs

The drugs, dosages, and sources used in this study were as follows: MDMA, 1.25, 2.5, 5, 10, and 20 mg/kg; MBDB, 1.25, 2.5, 5, 10, and 20 mg/kg; MMAI, 1.25, 2.5, 5, 10, and 20 mg/kg; and fenfluramine hydrochloride (2, 4, 6, and 10 mg/kg). All drugs were racemic and were synthesized in our laboratory (39,54). Drugs were dissolved in 0.9% saline and were injected intraperitoneally (IP) in a volume of 1 ml/kg, 30 min before conditioning.

Statistical Analysis

In the CPP paradigm, the difference in time spent on the drug-associated side between the post- and preconditioning tests indicated the change in preference induced by the drug. A positive difference reflected reward, a negative difference aversion. Data were analyzed by a two-way analysis of variance (ANOVA) for repeated measures on one factor. The factors were preconditioning–postconditioning test and treatments. In case of a significant effect of dose, the Neuman–Keuls procedure was used for a posteriori comparisons between doses.

For HPLC-EC assays, the concentrations of DA and DOPAC were determined using Dynamax Methods Manager software implemented on an Apple Macintosh SE computer. All comparisons used an ANOVA followed by a post hoc comparison as embodied in the computer program EPISTAT (EPISTAT Services, Richardson, TX).

RESULTS

Figure 1 shows the degree of CPP to MDMA, MBDB, fenfluramine, and MMAI. In comparison with the preconditioning test, the time spent by animals in the compartment that had been previously associated with injection of different

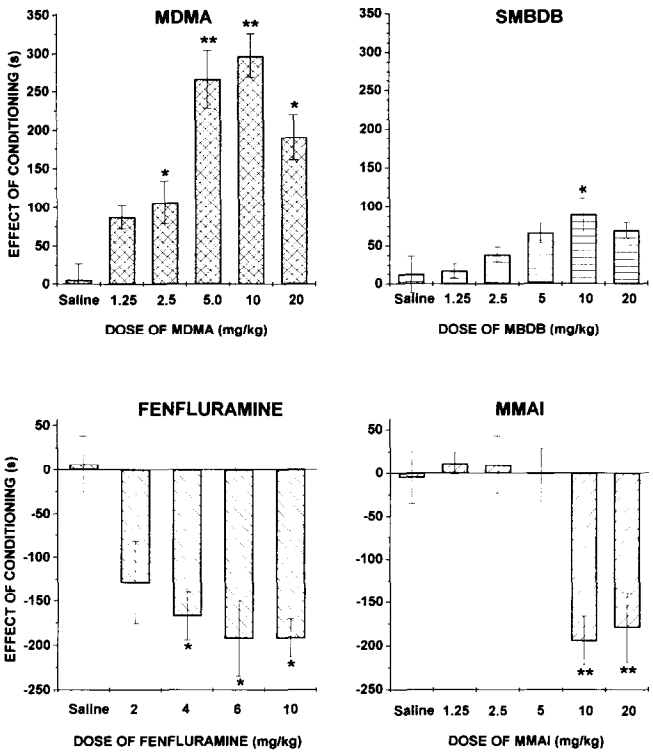

FIG. 1. Conditioned place preference established by various doses of MDMA (upper-left panel), MBDB (upper-right panel), fenfluramine (lower-left panel), and MMAI (lower-right panel). Data represent means ± SEM of the difference in the time spent in the nonpreferred compartment (paired with a drug) between the post- and preconditioning tests. Each value represents the mean of five rats. *$p < 0.05$, **$p < 0.001$, compared with the saline-treated group.

doses of MDMA (1.25, 2.5, 5, 10, and 20 mg/kg, IP) was significantly prolonged in the postconditioning test [$F(5, 24) = 11.27$; $p < 0.0001$]. The significant differences between the MDMA-treated groups indicated a dose dependence of the place preference. The lowest dose of MDMA (1.25 mg/kg) did not produce a significant effect in this paradigm. However, CPP induced by higher doses (2.5, 5, and 10 mg/kg) of MDMA was significantly different from the saline-injected group ($p < 0.05$) and from each other ($p < 0.05$). The effect of the highest 20-mg/kg dose of MDMA was significantly distinct from control ($p < 0.01$) but not from the other MDMA doses. The behavior of control animals was not changed by the training procedure ($p = 0.06$; NS).

MBDB, the α-ethyl homologue of MDMA, like the parent compound, produced CPP [$F(5, 24) = 3.12$; $p < 0.02$]. The effect induced by MBDB in this paradigm was dose dependent but about 2.5-fold weaker than the CPP produced by similar doses of MDMA. Only the 10-mg/kg dose of MBDB caused a significant difference in the conditioning effect compared with control ($p < 0.05$).

Fenfluramine evoked opposite effects from MDMA, causing place aversion. In this case, the conditioning effects induced by the four tested doses of fenfluramine (2, 4, 6, and 10 mg/kg) were independent of dose [$F(4, 20) = 2.33$, NS]. Nevertheless, fenfluramine at 4, 6, and 10 mg/kg produced significant place aversion, in comparison with control [$F(2, 12) = 7.52$; $p < 0.008$].

The time spent by the rats in the nonpreferred compartment that had been previously associated with injection of different doses of MMAI (1.25, 2.5, 5, 10, and 20 mg/kg, IP) was significantly reduced in the postconditioning test, in comparison with the preconditioning test [$F(5, 24) = 8.10$; $p < 0.001$]. Only the highest doses of MMAI (10 and 20 mg/kg) evoked significant place aversion vs. the saline-treated group ($p < 0.01$).

Figure 2 shows the time-course change in extracellular DA (upper panel) and DOPAC (lower panel) from microdialysis perfusates in the nucleus accumbens following peripheral administration of MDMA (6.3 mg/kg), MBDB (7.0 mg/kg), fenfluramine (7.7 mg/kg), and MMAI (6.2 mg/kg). A significant elevation over the basal level of DA was produced only by MDMA, from 40 min postinjection. This increase had not reached a maximum even 100 min after drug administration. MDMA also decreased DOPAC in the nucleus accumbens, but this reduction was not statistically significant.

No difference in extracellular DA or DOPAC was seen after injection of MBDB, fenfluramine, or MMAI. The first result is consistent with other studies. For example, in an earlier report (11,41), whole brain DA levels measured 3 h after MBDB were not significantly elevated.

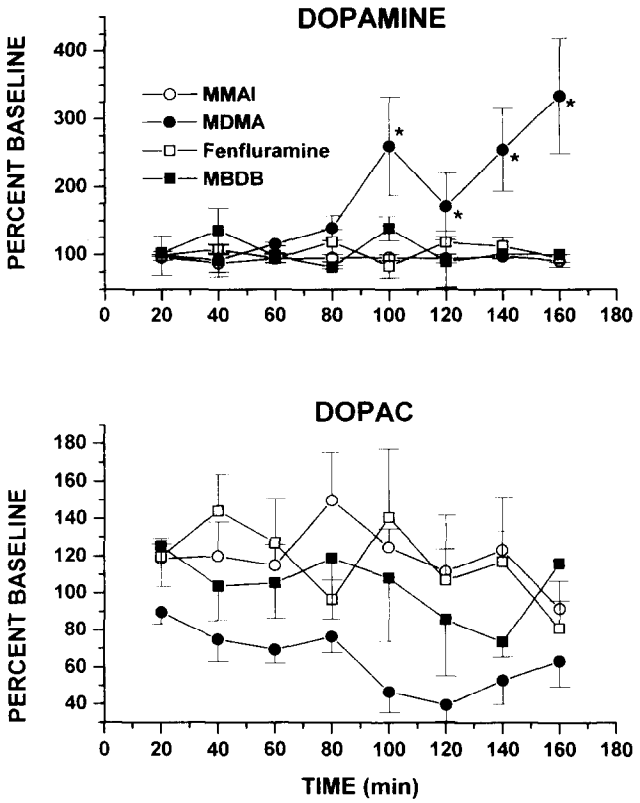

FIG. 2. Effects of MDMA, MBDB, fenfluramine, and MMAI on the extracellular concentration of dopamine (upper panel) and its metabolite, DOPAC (lower panel) in dialysates collected from rat nucleus accumbens as measured by in vivo microdialysis. Each value represents the mean ± SE of five rats expressed as the percent of basal concentrations for each rat. *$p < 0.01$, different from other groups. Dialysate samples were collected every 20 min. After the equilibration period, two baseline samples were collected, followed by a saline injection ($T = 0$ min). Three more samples were then collected at $T =$ 20, 40, and 60 min before treatment with MDMA, MBDB, fenfluramine, or MMAI ($T = 60$ min).

DISCUSSION

The experiments demonstrate that injections of various doses of MDMA or its α-ethyl homologue MBDB produce a CPP. These results agree with earlier reports showing that MDMA has rewarding properties that can be bestowed on previously neutral stimuli which can subsequently act as conditioned incentive stimuli in the absence of primary reward (6–8,69). Both 5-HT and DA have been identified as neurotransmitters most likely involved in the actions of MDMA-like compounds (31,36,67). Numerous studies show that MDMA causes the release of neuronal DA, which is probably critical to its reinforcing effect. Although in other studies MDMA produced a CPP that was attenuated by naltrexone, an opioid antagonist, and by MDL 72222, a 5-HT_3 receptor antagonist (6,8), opioidergic systems and 5-HT_3 receptors can also modulate the release of DA (20,30,34), and this seems to be a likely explanation for those effects.

Activation of striatal dopaminergic pathways has been implicated as the cause of stereotypic behavior seen after amphetamine administration, whereas mesolimbic DA areas may be involved in the reinforcing properties and euphoria-inducing effects of MDMA (8,76). We report here that MDMA at a dose producing a maximal effect in the CPP test is able to elevate extracellular DA in rat nucleus accumbens, measured using in vivo microdialysis in awake, freely moving rats.

One of the problems in interpreting in vivo effects of drugs is the possible involvement of active metabolites. MDMA is an example of such a drug, as it is converted to an active metabolite, MDA (24,40). This is particularly true in the present case, because the effect of MDMA on rat striatal DA is maximal at 60 min (52,53) or between 60 and 90 min (31) following drug administration, only returning to control levels by about 4 h. In another study, significant elevation of DA in the accumbens was observed 100 min after MDMA adminis-

tration, but DA continued to increase and did not reach a maximum until 3 h after drug (83). Our in vivo microdialysis data are in agreement with this latter study, where we observed a significant increase at 40 min after drug, with a continuing rise in DA over the next several hours. Although it has been reported that the increase in rat striatal DA by MDMA was due to the parent compound, with little contribution from the metabolite (32), it is possible that an active metabolite could be contributing to the prolonged elevation of DA in the accumbens.

In the course of structure-activity relationship studies, we originally synthesized MBDB, the α-ethyl homologue of MDMA (54). MBDB appears to lack the dopaminergic component present in the actions of MDMA, but does not differ in its ability to release 5-HT (54,57). MBDB produced a CPP, but this effect was significant only at the 10-mg/kg dose. Thus, the CPP effect evoked by MBDB was more than 2.5-fold weaker than that of MDMA. This is consistent with our working hypothesis that the dopaminergic effects of MDMA and MDMA-like compounds are responsible for their rewarding properties.

In contrast to the effects seen with MDMA, no difference in extracellular DA or DOPAC in rat nucleus accumbens was seen after injection of MBDB. Nash and Nichols (53) reported similar results using in vivo microdialysis in the striatum, where MDMA induced a nearly sixfold increase in striatal DA efflux compared with controls, whereas MBDB appeared to produce only a very slight and nonsignificant increase at one time point (60 min). In another report, rat cortical DA was not elevated 3 h after treatment with 25 mg/kg of MBDB, whereas 20 mg/kg of MDMA increased cortical DA by about threefold (41). However, 5 mg/kg of MBDB appeared to produce a slight but nonsignificant increase in cortical DA 40 min following treatment (11). Thus, although it may be possible that MBDB produces slight increases in DA in some brain regions, this response is clearly not robust, as is the case with MDMA. In the present study, the ability of MBDB to induce place preference was clearly inferior to that of MDMA. In that respect, the in vivo microdialysis result is consistent with the CPP data.

The in vivo microdialysis data do not, however, provide a basis for explaining the fact that MBDB did induce place preference, albeit weakly. Although there may be other explanations for this result, the one that seems most parsimonious with these and previous studies is as follows. If in fact reward phenomena are related to dopaminergic transmission in the nucleus accumbens, as is widely believed (15,16,43,75), a relatively small increase of DA efflux in this area may be sufficient to induce place preference. MBDB is a much weaker in vitro releaser of nonvesicular DA than is MDMA or its active metabolite MDA (38).

In contrast to the effect of MDMA and MBDB, fenfluramine and MMAI evoked place aversion, and neither fenfluramine nor MMAI increased extracellular DA in the accumbens. Similarly, there appear to be no data suggesting that these compounds have significant dopaminergic activity. Davis and Parker (21) reported that fenfluramine induced place aversion at doses ranging from 2.5–10 mg/kg. Our data confirm those earlier results, demonstrating a significant and similar intensity of place aversion for fenfluramine at doses of 4, 6, and 10 mg/kg. However, the aversive properties of fenfluramine in the CPP paradigm were independent of dose in these experiments. A similar effect was observed with the selective 5-HT releaser, MMAI. The aversive effect of MMAI was observed only at 10 and 20 mg/kg, as smaller doses of MMAI produced neither a place preference nor a place aversion. Callaway et al. (12) also reported similarities in patterns of locomotor activity between MMAI and fenfluramine. It has been reported, however, that the rewarding and aversive properties of several drugs are independent of their effect on locomotor activity (13).

A role for serotonin in drug reward processes has been suggested in studies in which pretreatment with drugs affecting serotonergic transmission was used to influence self-administration or drug-induced CPP (45,58). From the available data, it is impossible to formulate conclusions as to whether serotonin facilitates or inhibits drug reward processes. Based on the ability of both fenfluramine and MMAI to induce release of serotonin, however, it is tempting to speculate that this effect may somehow attenuate basal catecholaminergic neurotransmission.

Although fenfluramine and MMAI had similar aversive properties in this study, a striking difference does exist between these two compounds. Whereas high doses of fenfluramine can produce long-term deficits in markers of central serotonergic function (3,10,37,42,51,81,84), MMAI appears to lack such effects, even following repeated dosing (39).

In conclusion, the affective properties of MDMA and MMAI are significantly different as reflected in the CPP/aversion paradigm; MDMA produces CPP, whereas MMAI produces place aversion. It is our working hypothesis that the human psychopharmacology of MDMA and MBDB is the result of a combined action of each drug in both serotonergic and catecholaminergic pathways [see also (67)]. The lack of any significant in vitro or in vivo effect of MMAI on DA or norepinephrine (38) systems and the results presented here might thus be indicators that its human psychopharmacology may be more similar to fenfluramine than to MDMA. Because phentermine-fenfluramine drug combinations have recently been used to treat cocaine abuse and alcohol addiction in humans (25,74), MMAI may prove to be a useful nonneurotoxic serotonergic alternative compound with similar therapeutic potential.

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