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ORIGINAL INVESTIGATION

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Serotonin synthesis inhibition reveals distinct mechanisms of action for MDMA and its enantiomers in the mouse

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Abstract *Rationale:* Drug challenges in "intact" and *p*-chlorophenylalanine (*p*-CPA)-treated animals can be used to distinguish agents that act as direct serotonin (5-HT) agonists from agents that function as 5-HT releasers. *Objectives:* The objective of the study was to investigate the effect of *p*-CPA treatment on the capacity of racemic 3,4-methylenedioxymethamphetamine (MDMA) and its stereoisomers to induce the head twitch response, hyperthermia, and locomotor stimulation in mice. *Methods:* Pretreatments with either 100 mg/kg *p*-CPA or equivolume saline were administered for three consecutive days. The following day, mice were either euthanized (to quantify 5-HT tone), tested with various doses of racemic MDMA or one of its enantiomers in the head twitch assay, or challenged with 32 mg/kg racemic MDMA or one of its enantiomers, while temperature and locomotor activity were monitored via radiotelemetry. *Results:* *p*-CPA reduced cortical 5-HT turnover by >70% without altering dopamine turnover. Racemic MDMA did not induce a significant head twitch response in intact or *p*-CPA-treated mice. S(+)-MDMA and R(−)-MDMA elicited similar head twitch curves in intact mice; *p*-CPA treatment attenuated this response when induced by S(+)-MDMA but not when elicited by R(−)-MDMA. Neither the hyperthermic nor locomotor-stimulant effects of racemic MDMA were altered by *p*-CPA treatment. The hyperthermic effects, but not the locomotor-stimulant effects, of S(+)-MDMA were attenuated in mice treated with *p*-CPA. R(−)-MDMA did not alter core temperature or induce significant locomotor stimulation in intact or *p*-CPA-treated mice. *Conclusions:* The effects of S(+)-MDMA on core temperature and head twitch behavior are consistent with a mechanism involving 5-HT release, whereas the effects of R(−)-MDMA on head twitch behavior are consistent with a direct agonist mechanism of action. The actions of the racemate on core temperature and locomotor activity likely involve a combination of 5-HT release and direct agonism at 5-HT receptors.

Keywords *p*-CPA · Head twitch response · Locomotor activity · Hyperthermia

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Introduction

Few studies have directly compared the behavioral and pharmacological effects of 3,4-methylenedioxymethamphetamine (MDMA) and its enantiomers, but those that have typically report differences. For example, Battaglia and DeSouza (1989) conducted ex vivo radioligand binding experiments which revealed an asymmetry in the binding profiles of the MDMA enantiomers, such that S(+)-MDMA had higher affinity for presynaptic serotonin (5-HT) transporters, whereas R(−)-MDMA had higher affinity for postsynaptic 5-HT receptors. Similarly, in rat synaptosomes, S(+)-MDMA was demonstrated to be more potent than R(−)-MDMA in terms of 5-HT release (Nichols et al. 1982), and later studies of the blockade of 5-HT, dopamine (DA), and norepinephrine (NE) reuptake revealed a modest stereoselectivity, with S(+)-MDMA producing slightly (two- to fourfold) lower IC_{50} values for all three monoamines (Nichols 1986). With regards to in vivo experiments, we have previously compared the MDMA enantiomers in terms of their capacity to engender contingent responding in rhe-

sus monkeys (Fantegrossi et al. 2002), to induce lethality, hyperthermia, and locomotor stimulation (Fantegrossi et al. 2003), as well as the head twitch response in the mouse (Fantegrossi et al. 2004a). The results of these studies were generally consistent with the binding data previously described. For example, the reinforcing effects of R(−)-MDMA in the rhesus monkey were more susceptible to disruption via the selective 5-HT_{2A} antagonist (+)-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol (M100907, formerly MDL100907) than were those of the racemate or S(+) enantiomer (Fantegrossi et al. 2002). A similar pattern of effects was obtained for the lethal effects of these compounds in singly housed mice (Fantegrossi et al. 2003). These results, among others, have led us to conceptualize S(+)-MDMA as a 5-HT "releaser," whereas R(−)-MDMA may be more akin to a direct 5-HT agonist, although both isomers overlap in some endpoints and certainly exert other important pharmacological actions as well (Battaglia et al. 1988; Acquas et al. 2001; Fischer et al. 2001; Bunzow et al. 2001).

Past research has attempted to behaviorally distinguish drugs that act as direct 5-HT agonists from drugs that act as 5-HT releasers by comparing the effects of these compounds in "intact" and 5-HT-depleted animals. There are multiple agents that deplete 5-HT, and the mechanisms of action for these agents are varied. Correspondingly, results obtained under different depletion regimens are not necessarily consistent, even when depletion magnitude is equalized (see, for example, Joseph and Appel 1977). And yet, across several behavioral assays, the effects of 5-HT releasers are attenuated in depleted animals, whereas the effects of direct agonists are either potentiated (when depletion results in receptor supersensitivity) or not altered (when depletion does not change receptor sensitivity). Depletion in the absence of altered sensitivity to the effects of 5-HT agonists has often been accomplished via repeated treatment with the 5-HT synthesis inhibitor *para*-chlorophenylalanine (*p*-CPA). *p*-CPA acts as an irreversible inhibitor of tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis. A representative and relevant finding in this regard was reported by Balsara et al. (1986), wherein mice treated with 100 mg/kg *p*-CPA for four consecutive days displayed an attenuated head twitch response when challenged with the 5-HT releaser fenfluramine, although the effects of the direct 5-HT agonist ergometrine on head twitch behavior were not altered in these animals.

Locomotor-stimulant effects of drugs have been strongly linked to increased dopamine (DA) neurotransmission in specific brain regions (Wise and Bozarth 1987; Piazza et al. 1991). However, studies investigating the neurochemical correlates of the locomotor-stimulant effects of racemic MDMA (Nair and Gudelsky 2004; Bengel et al. 1998) and S(+)-MDMA (Bankson and Cunningham 2002; Bubar et al. 2004) in rodents have shown that the dopaminergic effects of these agents are likely to be indirectly mediated via serotonergic mechanisms. As such, prior treatment with *p*-CPA might be expected to disrupt the locomotor effects of each compound. Similarly, we have previously shown that the hyperthermic effects of racemic MDMA and S(+)-MDMA are sensitive to serotonergic antagonism (Fantegrossi et al. 2003, 2004a) in the mouse, whereas others have reported similar findings in the rat (Schmidt et al. 1990).

Herein we report the effects of racemic MDMA and its enantiomers on head twitch behavior, locomotor activity, and hyperthermia in intact and *p*-CPA-treated mice. The effects of MDMA on these endpoints have been previously reported, but the effects of *p*-CPA treatment on MDMA-induced head twitches, locomotor stimulation, and hyperthermia have not heretofore been studied in the mouse. To quantify the effects of *p*-CPA treatment on 5-HT tone, a group of MDMA-naive mice was euthanized at the end of the *p*-CPA regimen. Cortical tissue concentrations of 5-HT, its major metabolite 5-hydroxyindoleacetic acid (5-HIAA), DA, and its major metabolites homovanilic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were quantified via high-pressure liquid chromatography (HPLC).

Materials and methods

Animals

Male NIH Swiss mice (Harlan Sprague Dawley Inc., Indianapolis, IN) weighing 20–30 g were housed 12 animals per 44.5×22.3×12.7 cm Plexiglas cage prior to use in drug-elicited head twitch experiments or surgical implantation of radiotelemetry probes. Mice were housed in a temperature-controlled room maintained at an ambient temperature of 22±2°C at 45–50% humidity during all phases of study. Lights were set to a 12-h light/dark cycle. Animals were fed with standard rodent chow (Laboratory Rodent Diet #5001, PMI Feeds, Inc., St. Louis, MO) and water ad libitum until immediately before testing. Animals were not used in experiments until at least 2 days after arrival in the laboratory. Each animal was used only once and was sacrificed immediately after use.

Procedure

p-CPA treatment regimen and quantification of monoamines

Mice were injected intraperitoneally (i.p.) with 100 mg/kg *p*-CPA or equivolume saline for three consecutive days. On the fourth day, mice were tested in one of the assays below or euthanized so that the extent of 5-HT depletion could be quantified. For these latter animals, brains were rapidly removed on ice following cervical dislocation and decapitation. Cortex was dissected freehand using blunt curved microforceps. Tissue samples were immediately placed into cryovials on dry ice and stored at −70°C until assay.

For tissue determinations, each sample was transferred to 400 μl ice-cold 0.1 N perchloric acid containing *N*-methyl-5-HT and 3,4-dihydroxybenzylamine as internal standards (for indoleamines and catecholamines, respectively). The tissue was sonicated in this solution and centrifuged at

23,000×*g* for 20 min at 4°C. A portion of the supernatant (50 ml) was removed and analyzed by high-pressure liquid chromatography (HPLC) to determine the concentration of DOPAC, HVA, DA, 5-HT, and 5-HIAA. The column employed was from Bioanalytical Systems (BAS: West Lafayette, IN; Phase II ODS, 3 mM, 100×3.2 mm). The on-line degassed mobile phase consisted of an 8% solution of acetonitrile containing 0.6% tetrahydrofuran, 0.1% diethylamine, 0.025 mM ethylenediaminetetraacetic acid, 2.3 mM 1-octane-sulfonic acid, 30 mM sodium citrate, and 13.7 mM sodium dihydrogen phosphate (final pH 3.1) and was delivered at 600 ml/min. Tissue pellets were saved for protein determination using the DC Protein Assay (Bio-Rad, Hercules, CA) with bovine serum albumin as the protein standard. In this system, optical densities are automatically converted into milligram units derived from a standard curve. All protein values are based upon the mean of three replicates.

Chromatograms were recorded using a DA-5 data acquisition analog to digital interface module coupled to an LC-4C electrochemical detector (BAS). Postseparation signals were derived from a 2-mm glassy-carbon working electrode whose potential was set at 600 mV vs a Ag/Ag Cl reference. Peak height quantification involved dividing the peak height of the unknown by that of the internal standard and referring this ratio to external standards. Because absolute tissue levels reflect both intra- and extrasynaptic neurochemical concentrations, calculated turnover is presented as a more accurate reflection of neurochemical content in each tissue sample. Measurements of turnover are expressed as the ratio of tissue concentration (in ng/mg protein) of the primary acidic metabolites (DOPAC and HVA or 5-HIAA) to the parent amine (DA or 5-HT). Samples from all animals were processed in parallel on the same day for each brain region.

Drug-elicited head twitch response

On experimental days, mice were weighed, marked, and returned to the home cage. Doses were then calculated and prepared for injection. Individual animals were subsequently removed from the home cage, injected i.p. with saline, then placed into a 15.24×25.40×12.70 cm Plexiglas mouse cage. Ten minutes after the initial injection, mice were injected with various doses of racemic MDMA, S(+)-MDMA, R(−)-MDMA, or saline and returned to the small observation cage. Five minutes after this second injection, a camera mounted above the observation cage began recording behavior and continued to do so for 10 min. Videotapes were later scored for drug-elicited head twitches, here defined as a rapid rotational jerk of the head that is not contiguous with any grooming or scratching behaviors, by two blind observers (e.g., Fantegrossi et al. 2004a). All head twitch experiments were conducted in the colony room at an ambient temperature of 22±2°C, and neither food nor water was available during the tests.

Core temperature and locomotor activity experiments

Following appropriate anesthetization with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), the abdominal area of each mouse was shaved and sanitized with iodine swabs. A rostral-caudal cut 1.5 cm in length was made with skin scissors, providing access to the intraperitoneal cavity. A cylindrical glass-encapsulated radiotelemetry probe (model ER-4000 E-Mitter, Mini Mitter Co., Inc., Bend, OR, USA) was then inserted, and the incision was closed using absorbable 5–0 chromic gut suture material. Surgeries were carried out at least 7 days before initiation of the *p*-CPA depletion regimen, allowing time for incisions to heal and for the mice to recover normal body weights. Following surgery, all implanted mice were individually housed in 15.24×25.40×12.70 cm Plexiglas mouse cages for the duration of all temperature and locomotor activity experiments. Implanted transmitters produced activity- and temperature-modulated signals which were sent to a receiver (ER-4000 Receiver, Mini Mitter Co., Inc.) underneath each mouse cage. On experimental days, mice were weighed, marked, and returned to their individual cages. MDMA doses were then calculated and prepared for injection. Animals were subsequently removed from their cage and injected i.p. with 32 mg/kg racemic, S(+)-, R(−)-MDMA, or equivolume saline and returned to their cage, where temperature and locomotor activity data were collected at 5-min intervals and processed simultaneously by the Vital View data acquisition system (Mini Mitter Co., Inc.) for 3 h. This dose was chosen based on previous studies (Fantegrossi et al. 2003) and represents a nonlethal dose that elicits significant hyperthermia and locomotor stimulation for racemic and S(+)-MDMA.

Data analysis

For the head twitch experiments, data are presented as mean ±SEM and were compared to values obtained from equivolume saline controls (n=6, temperature data not shown) using one-way ANOVA (one-tailed) and Tukey's post hoc tests. Neurochemical data were compared by Student's *t*-tests. All statistical tests were performed using commercially available software, and significance was judged at $P<0.05$.

Drugs

Racemic MDMA and its enantiomers were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC) and were dissolved in physiological saline prior to injection. M100907 was synthesized at the Laboratory of Medicinal Chemistry at the National Institutes of Diabetes, Digestive and Kidney Disorders at the National Institutes of Health (Bethesda, MD) and dissolved in sterile water and 0.5 N HCl. *p*-CPA was purchased from Tocris Cookson (Ellisville, MO) and was dissolved in 0.01 N NaOH and sterile water to a final pH of

8. All injections were administered i.p. in a volume equal to body weight (g)/100.

Results

p-CPA treatment regimen and quantification of monoamines

Treatment with *p*-CPA reduced cortical 5-HT turnover by greater than 70% (critical t=2.33, 95% confidence interval for difference of means 0.012–1.98, P<0.05). This effect was selective for 5-HT systems, as DA turnover was not altered by *p*-CPA treatment (Fig. 1).

Drug-elicited head twitch response

Following saline injection, approximately one head twitch was noted during the 10-min observation period for both intact and *p*-CPA-treated animals; there was no statistical difference between the results obtained with saline across these groups, so data were collapsed into a single point (filled triangles, all panels of Fig. 2). Racemic MDMA induced few head twitches in either intact mice or in animals with reduced 5-HT turnover (Fig. 2, top panel). The maximal number of twitches elicited by racemic MDMA at all doses was never different from that observed in saline control animals for either intact or depleted mice (P>0.05 for both groups). In contrast to the racemate, S(+)-MDMA induced an inverted U-shaped dose–effect curve across the dose range tested in both groups of mice (Fig. 2, middle panel). For intact mice, maximal twitches were elicited at a dose of 0.32 mg/kg S(+)-MDMA, and this effect was significantly greater than that observed in saline controls (p=5, q=5.79, P<0.05). Importantly, *p*-CPA treatment shifted the S(+)-MDMA head twitch curve down and to the right, and although the shape of the curve was preserved, no dose of S(+)-MDMA elicited significantly more head twitch behavior than saline in these animals with reduced 5-HT turnover (P>0.05). Similar to S(+)-MDMA, R(−)-MDMA induced an inverted U-shaped dose–effect curve in both intact and *p*-CPA-treated mice (Fig. 2, bottom panel). Interestingly, *p*-CPA treatment did not alter the effects of R(−)-MDMA on head twitch behavior. In both groups of animals, 1.0 mg/kg R(−)-MDMA elicited significantly more head twitches than did saline (p=4, q=9.16 for intact mice; p=4, q=4.89 for mice with reduced 5-HT turnover; P<0.05 for both groups).

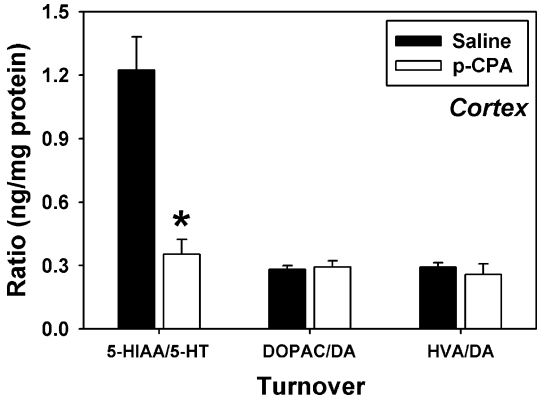

Fig. 1 Neurochemical content in cortex following 3-day treatment with saline (*filled bars*) or *p*-CPA (*open bars*.) *Asterisks* indicate significance (P<0.05) by Student's *t*-test. See text for more information

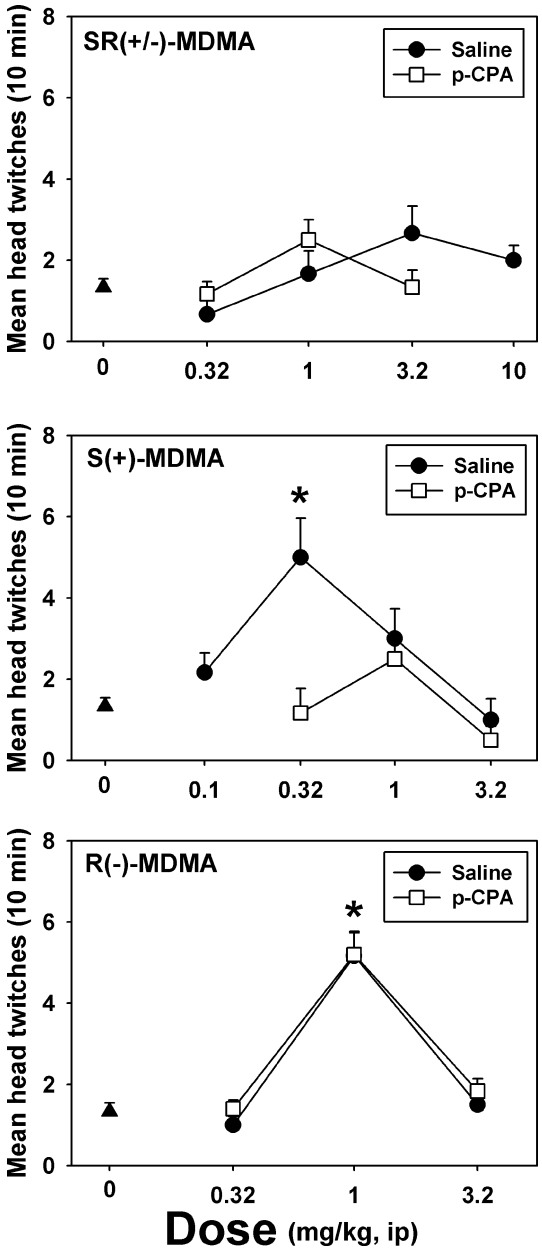

Fig. 2 Effects of racemic MDMA (*top*), S(+)-MDMA (*middle*), and R(−)-MDMA (*bottom*) on head twitch behavior in intact (*filled symbols*) and 5-HT-depleted (*open symbols*) mice. Each *point* represents the mean±SEM (n=6 mice per dose). *Abscissae*: MDMA dose (mg/kg, i.p.). *Ordinates*: Mean head twitches/10 min. *Asterisks* indicate significant differences from saline controls (P<0.05) by one-way ANOVA and Tukey's post hoc tests

Core temperature and locomotor activity experiments

Following saline injection, no systematic changes were observed in core temperature during the observation period; there was no difference between the results obtained with saline in intact or *p*-CPA-treated mice, so data were collapsed across groups and presented as a range of minimum and maximum temperatures observed over the 180-min recording period (gray region, all left-hand panels of Fig. 3). Racemic MDMA rapidly induced an increase in core temperature in intact mice; by 30-min postinjection, core temperature had risen 2°C. This hyperthermic effect gradually recovered, and baseline temperatures were re-established by 120 min after injection. Prior treatment with *p*-CPA did not alter the hyperthermic effects of racemic MDMA (Fig. 3, top left panel). Similar to the racemate, S(+)-MDMA induced a hyperthermic response in both groups of animals tested, but this effect was notably blunted in mice with reduced 5-HT turnover. Prior treatment with *p*-CPA resulted in an attenuation of the magnitude and time course of S(+)-MDMA-induced hyperthermia (Fig. 3, middle left panel). In contrast to both the racemate and the S(+) enantiomer, R(−)-MDMA did not alter core temperature in either group of mice (Fig. 3, bottom left panel).

Locomotor activity was greatest in the 15-min period immediately following saline injection, but no orderly changes were observed at later time points. There was no difference between the locomotor results obtained with saline in intact or *p*-CPA-treated mice, so data were collapsed across groups and are presented as a range of minimum and maximum activity counts observed over the 180-min recording period (gray region, all right-hand panels of Fig. 3). Racemic MDMA elicited a profound and long-lasting locomotor stimulation in intact mice. This locomotor-stimulant effect gradually recovered by 180 min after injection. Prior treatment with *p*-CPA did not alter the locomotor-stimulant effects of racemic MDMA (Fig. 3, top right panel). S(+)-MDMA induced a similar, though less pronounced, locomotor-stimulant effect in both groups of animals tested. Prior treatment with *p*-CPA did not alter the locomotor-stimulant effects of S(+)-MDMA (Fig. 3, middle right panel). R(−)-MDMA elicited a modest and brief increase in locomotor activity in both groups of mice. This effect was apparent only in the first 15-min period following injection.

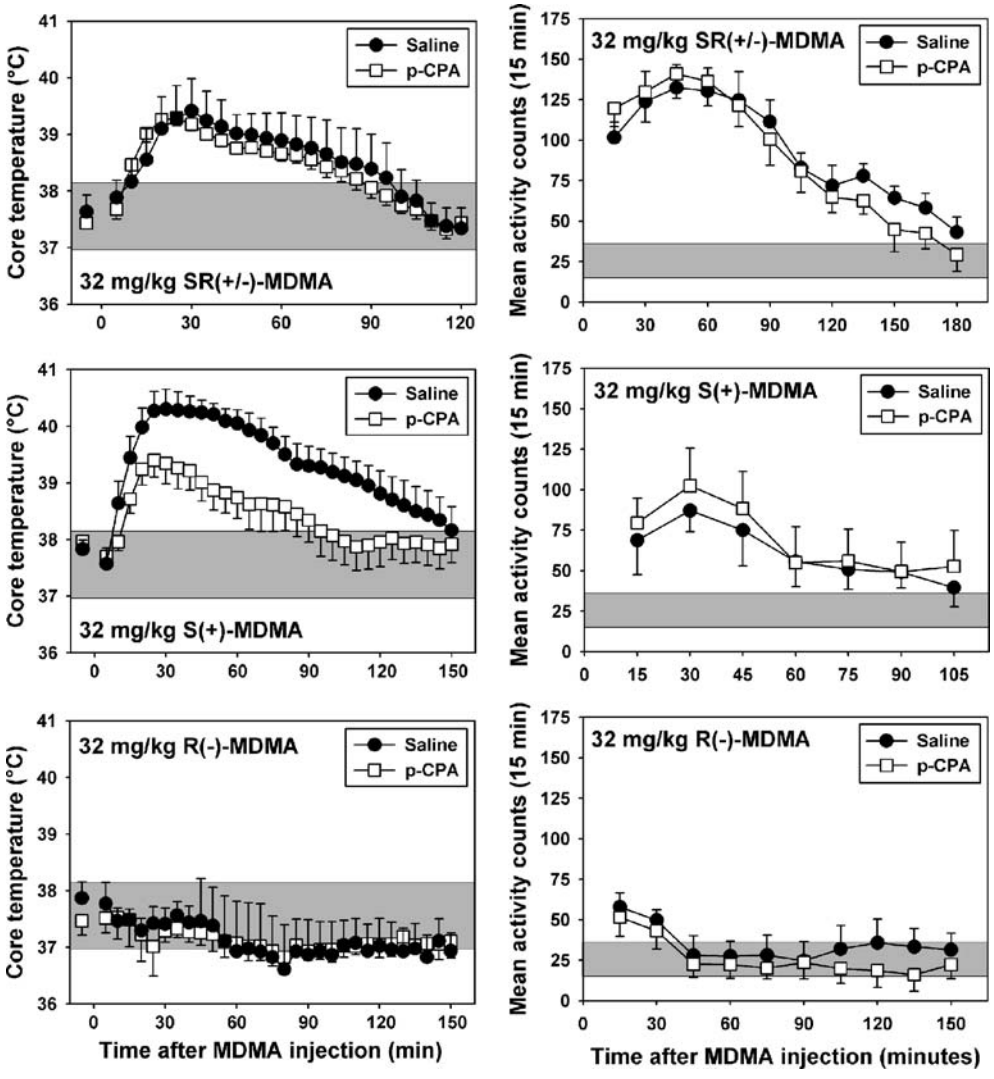

Fig. 3 *Left panels*: Effects of racemic MDMA (*top*), S(+)-MDMA (*middle*), and R(−)-MDMA (*bottom*) on core temperature in intact (*filled symbols*) and 5-HT-depleted (*open symbols*) mice. Each *point* represents the mean±SEM (*n*=6 mice per dose). *Abscissae*: Time after injection (h). *Ordinates*: Core temperature (°C). The gray region between approximately 37 and 38°C represents the normal range of rodent core temperature measured over a 3-h period following equivolume saline injection. *Right panels*: Effects of racemic MDMA (*top*), S(+)-MDMA (*middle*), and R(−)-MDMA (*bottom*) on locomotor activity in intact (*filled symbols*) and 5-HT-depleted (*open symbols*) mice. Each *point* represents the mean±SEM (*n*=6 mice per dose). *Abscissae*: Time after injection (h). *Ordinates*: Activity counts. The gray region between ~15 and 35 counts represents the normal range of rodent locomotor activity measured over a 3-h period following equivolume saline injection

Prior treatment with *p*-CPA did not alter the locomotor-stimulant effects of R(−)-MDMA (Fig. 3, bottom right panel).

Discussion

In the present studies, prior treatment with *p*-CPA did not alter the effects of R(−)-MDMA on head twitch behavior, and no effects on core temperature or locomotor activity were observed in intact or treated animals. The results of the head twitch assay suggest that R(−)-MDMA either functions as a direct 5-HT agonist (at least, in terms of this particular endpoint) or that this effect is not serotonergically mediated. The later possibility does not seem likely given the results of previous antagonist studies where R(−)-MDMA-induced head twitches were attenuated by two 5-HT_2 antagonists (Fantegrossi et al. 2004a). Further evidence for the mediation of the behavioral effects of R(−)-MDMA by 5-HT systems comes from rhesus monkey studies where contingent responding for R(−)-MDMA was selectively attenuated by two 5-HT_2 antagonists (Fantegrossi et al. 2002). However, in agreement with previous reports, R(−)-MDMA has only weak effects on locomotor activity in the intact mouse and does not appear to induce hyperthermia up to doses that result in some lethality (Fantegrossi et al. 2003). One possible explanation for the general lack of locomotor-stimulant properties for this compound comes from in vitro studies of transporter-mediated monoamine release. One such study has shown that R(−)-MDMA is at least tenfold less potent than racemic and S(+)-MDMA at dopamine transporters and approximately five times less potent than racemic and S(+)-MDMA at norepinephrine transporters (Setola et al. 2003). Similarly, pilot experiments with the structurally similar direct 5-HT_2 agonist 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI) in the mouse also induced a head twitch response that was insensitive to *p*-CPA treatment (Fantegrossi et al. 2004b) but failed to induce locomotor stimulation or hyperthermia at doses up to 30 times higher than those required to elicit significant head twitch behavior (unpublished results). Although 5-$HT_{2A/2C}$ agonists have previously been shown to induce hyperthermia in the rat (Mazzola-Pomieto et al. 1997), this lack of effects on core temperature may be a property of direct 5-HT_2 agonists in mice.

In contrast, the pattern of effects obtained with S(+)-MDMA seems consistent with this enantiomer acting via a mechanism dependent on endogenous 5-HT tone. That prior treatment with *p*-CPA attenuated head twitch behavior and hyperthermia suggests that these effects are mediated by S(+)-MDMA-stimulated 5-HT release. Interestingly, the locomotor-stimulant effects of S(+)-MDMA were not altered by 5-HT depletion. We have previously reported that the 5-HT_2 antagonist ketanserin, the selective 5-HT_{2A} antagonist M100907, and the serotonin selective reuptake inhibitor fluoxetine, at doses that attenuated the locomotor-stimulant effects of racemic MDMA, *potentiated* the locomotor-stimulant effects of S(+)-MDMA (Fantegrossi et al. 2003). These data, along with the presently reported differences regarding the effects of *p*-CPA treatment on the locomotor-stimulant effects of racemic vs S(+)-MDMA, are puzzling, as in vitro studies have demonstrated similar potencies for these compounds at dopamine and norepinephrine transporters (Setola et al. 2003). The pharmacological mechanisms underlying S(+)-MDMA-induced locomotor stimulation in mice clearly deserve further study.

Finally, the cluster of effects seen with racemic MDMA does not lend itself to a consistent interpretation in terms of 5-HT release and/or direct agonism. We have previously reported that both S(+)- and R(−)-MDMA induce head twitches in mice (Fantegrossi et al. 2004a) but present for the first time evidence that racemic MDMA does not elicit this behavior in the mouse. Why each of the individual isomers should elicit this behavior on its own, but not when administered in combination as racemic MDMA, is not readily apparent. One possibility has to do with the small potency difference observed between S(+)-MDMA and R(−)-MDMA and the biphasic nature of the dose–effect curves generated. These particular circumstances may result in reciprocal antagonism across the range of racemic MDMA doses presently tested. However, this possibility assumes that the enantiomers are evenly absorbed and distributed in the mouse following administration of racemic MDMA, and there is, as yet, no published evidence in this regard to either support or challenge this interpretation of the data.

Clearly, the hyperthermic effects of racemic MDMA are not dependent on endogenous 5-HT tone, implying that racemic MDMA affects core temperature either by acting as a direct 5-HT agonist or by impacting some other neurochemical system. This later possibility is unlikely given the previously described experiments wherein racemic MDMA-induced hyperthermia in the mouse was blocked by various serotonergic agents, including ketanserin, M100907, and fluoxetine (Fantegrossi et al. 2003), as well as the plant-derived aporphine, nantenine (Fantegrossi et al. 2004a). However, given that S(+)-MDMA appears to alter core temperature via a 5-HT release mechanism, and given that R(−)-MDMA does not affect core temperature at all, it is difficult to understand why racemic MDMA should elicit full hyperthermic effects in the *p*-CPA-treated mouse. It may be the case that active metabolites of racemic MDMA are responsible for the effects of this compound on core temperature. Indeed, similar explanations have been offered for the neurochemical depleting effects of racemic MDMA in the rat (Esteban et al. 2001).

In correspondence with the results previously described for S(+)-MDMA, the locomotor-stimulant effects of racemic MDMA were not altered by *p*-CPA treatment, despite the pronounced reduction in 5-HT turnover. This effect seems consistent with the supposition that racemic MDMA exerts its locomotor-stimulant effects via a direct agonist action, as we have previously shown that ketanserin, M100907, and nantenine all attenuated MDMA-induced locomotor activity at doses that did not alter spontaneous locomotor activity on their own (Fantegrossi et al. 2003, 2004a). However, similar effects were also obtained with the 5-HT selective reuptake inhibitor fluoxetine (Fantegrossi et al. 2003),

which does not seem consistent with a direct 5-HT agonist mechanism of action for the locomotor-stimulant effects of racemic MDMA. As with the hyperthermic effects, the profound locomotor-stimulant effects of racemic MDMA are difficult to reconcile given the effects of the individual isomers on this endpoint. The dose of racemic MDMA used in the present studies, 32 mg/kg, should be equivalent to 16 mg/kg of each isomer delivered together. The locomotor-stimulant effects of S(+)-MDMA are less than those of the racemate, and the effects of R(−)-MDMA are milder still. Therefore, something beyond simple additivity must therefore be invoked in order to explain the pronounced effects of racemic MDMA on locomotor activity in the mouse.

It seems likely that racemic MDMA and its enantiomers may differ in terms of affinity and efficacy across multiple receptor systems (although data to support this notion are few), and it may be plausible to speculate that the stereoisomers may, on occasion, interact with each other in complex ways in vivo. Indeed, as we have previously reported, the in vivo effects of racemic MDMA and its enantiomers may be distinguished not only in terms of quantitative differences, but also in terms of qualitative differences (Fantegrossi et al. 2003). Relatively few studies have directly compared the MDMA enantiomers across measures, yet it seems reasonable to suggest that the isomers have heterogeneous effects that manifest themselves across a wide range of endpoints, perhaps indicating important differences in their underlying pharmacology. In this regard, the differences herein noted between the effects of racemic MDMA and its enantiomers in intact and *p*-CPA-treated mice are illustrative as stereoselective effects were apparent in all assays. S(+)-MDMA appears to elicit hyperthermia and head twitch behavior via a mechanism involving 5-HT release but stimulates locomotor activity through an action that is not regulated by endogenous 5-HT tone. R(−)-MDMA seems to induce head twitches via direct agonist action at 5-HT receptors but has no pronounced effects on core temperature or locomotor activity. Racemic MDMA induces hyperthermia and locomotor stimulation through a mechanism that is not dependent on endogenous 5-HT tone but does not induce significant head twitch behavior. These results suggest that the pharmacological actions of MDMA and its enantiomers are likely to be mediated by different mechanisms across multiple measures in vivo. We would caution that any in vivo results obtained with the MDMA enantiomers may not be particularly informative with regards to the racemate and vice versa.

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