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Attenuation of the stimulant and convulsant effects of cocaine by 17-substituted-3-hydroxy and 3-alkoxy derivatives of dextromethorphan

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Abstract

Pharmacological evidence has suggested a role for both sigma and *N*-methyl-D-aspartate (NMDA) receptors in the behavioral stimulant effects of cocaine and its convulsant effects observed at higher doses. A series of dextromethorphan (DM) analogs with a range of affinities for sigma-1 binding sites and for the NMDA receptor ion channel were used to explore the contribution of these two mechanisms in controlling the stimulant and convulsant effects of cocaine. These compounds were potent and efficacious blockers of both stimulant and convulsant effects produced by acute cocaine administration in mice (cocaine 10 or 75 mg/kg ip for locomotor activity or convulsions, respectively). Generally, the DM analogs blocked these effects of cocaine at doses that did not display ataxic and sedative side effects as measured in the inverted screen test. In contrast to the high-affinity NMDA blockers, (+)-MK-801 (dizocilpine) and dextrorphan (DX), DM and analogs did not stimulate locomotor activity. There was no significant correlation between the affinities of the DM analogs for the sigma-1 or the phencyclidine (PCP) binding site and their potencies to produce behavioral effects on their own or to attenuate the behavioral or toxic effects of cocaine. The present study has identified a series of agents that have cocaine-blocking effects that appear to be distinct from that of classical sigma-1 receptor ligands and that of traditional uncompetitive NMDA receptor antagonists. These findings point to potentially novel pharmacological strategies for blocking cocaine stimulant and toxic effects.
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Keywords: Sigma-1; NMDA; PCP; Dextromethorphan; Cocaine; Locomotor activity; Seizures

1. Introduction

Dextromethorphan [(+)-3-methoxy-17-methylmorphinan; DM] has been used for over 30 years as a nonprescription cough suppressant and has shown a high margin of safety. However, there is substantial evidence that this compound can display a number of other potentially interesting and clinically relevant central effects, including anticonvulsant/neuroprotectant actions (Tortella et al., 1989, 1999). DM binds with low affinity (513–3500 nM) to the phencyclidine (PCP) site of the *N*-methyl-D-aspartate (NMDA) receptor (Murray and Leid, 1984; Klein and Musacchio, 1989; Newman et al., 1996). However, DM is metabolized in vivo to dextrorphan (DX), a compound with high affinity (23–460 nM) for the same PCP site (Franklin and Murray, 1992; Newman et al., 1996). Thus, some of the behavioral actions and neuropharmacological effects (including neuroprotective and anticonvulsant effects) of this compound have been attrib-

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uted to an uncompetitive blockade of the NMDA receptor ion pore. However, DM has been shown to bind with high affinity to at least two other sites in the rat brain, which may be relevant to its mechanism of action. One of these sites exhibits the pharmacological profile of the sigma-1 receptor while the other appears to be a high-affinity (20 nM), DM-specific binding site (Klein and Musacchio, 1992). The contribution of these sites to the behavioral and neurological effects of DM continues to be unresolved.

DM has been reported to alter some of the behavioral effects of cocaine (Karler and Calder, 1992; Kim et al., 1997; Pulvirenti et al., 1997). These effects have been attributed to the NMDA blocking properties of this compound. NMDA antagonists have been shown to prevent some of the acute behavioral actions of cocaine, including locomotor activation and seizures, as well as the sensitization to these effects observed after chronic cocaine administration (Derlet and Albertson, 1990; Witkin, 1993; Witkin and Tortella, 1991; Witkin et al., 1999; Rockhold et al., 1991; Seidleck et al., 1994; Brackett et al., 2000). However, it is not known whether the binding of DM to the sigma receptor could contribute to some of these effects since it has been established that sigma ligands are also able to block some of the acute behavioral effects of cocaine, including locomotor stimulation (Menkel et al., 1991; Witkin et al., 1993; Hascoet et al., 1995; Maj et al., 1996; McCracken et al., 1999b) and cocaine-evoked seizures (Witkin et al., 1993; Ritz and George, 1997; McCracken et al., 1999a).

Recently, a series of DM derivatives with improved selectivity for sigma-1 versus PCP binding sites was prepared (Newman et al., 1996). Binding affinities for sigma-1 receptors ranged from $K_i = 8$ to 8000 nM. However, none of these compounds exhibited significant affinity for the sigma-2 site (K_i ranging from 0.5 to >10 μM). In addition, these compounds displayed a broad range of moderate to low affinities ($K_i = 0.5–98$ μM) for the ion channel of the NMDA receptor (PCP binding site). Therefore, this series of compounds represents a useful tool (a) to investigate the relative contribution of sigma-1 and PCP binding sites to the behavioral actions of DM and its analogs, and (b) to further characterize the potential role of sigma-1 receptors in modulating the acute behavioral and toxic effects of cocaine. To this end, effects of these compounds on cocaine-induced locomotor stimulation and seizures were investigated. In addition, these drugs were evaluated for possible PCP-like behavioral and motor impairing effects (Ginski and Witkin, 1994). The parent compound DM and its primary metabolite, DX were also evaluated. In addition, a typical PCP site ligand, (+)-MK-801, and a selective sigma ligand, NPC 16377 were included in the study as reference compounds. NPC 16377 selectively binds to sigma receptors without significant affinity for other neurotransmitter receptors (Karbon et al., 1993).

2. Methods

2.1. Animals

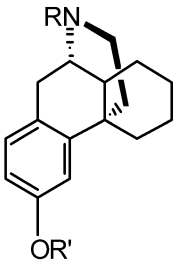
Experimentally naive, male Swiss Webster mice (Taconic Farms, Germantown, NY) between 6 and 10 weeks of age were housed four per cage in a temperature-controlled vivarium. All animals were acclimated to their home cages and to the light/dark cycle for at least 5 days prior to testing. Water and food were continuously available for the mice in their home cages. Experiments were conducted during the light phase of a 12-h light/dark cycle. The facilities in which the animals were maintained are fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and the studies described here were conducted in accordance with the Guide for Care and Use of Laboratory Animals of the NIH and adopted by NIDA in protocols approved by the institutional Animal Care and Use Committee.

2.2. Behavioral toxicity

Immediately prior to administration of cocaine and subsequent behavioral testing, mice were first assessed on the inverted screen test. The inverted screen test was used to assess one form of behavioral toxicity induced by the test compounds. In this test, compounds with sedative and/or ataxic properties produce dose-dependent increases in screen failures, whereas other classes of drugs (e.g. psychomotor stimulants) do not (Ginski and Witkin, 1994). Mice (at least eight per group) were pretreated with either vehicle or the test compound and were returned to their home cage for the appropriate pretreatment interval (see Drugs section below). They were then individually placed on a 14 × 14-cm wire mesh screen (0.8-cm screen mesh) elevated 38 cm above the ground. After slowly inverting the screen, the mice were tested during a 2-min trial for their ability to climb to the top. Mice not climbing to the top (all four paws on upper surface) were counted as a failure. Results were expressed as a toxic dose (TD_{50}) value. Each TD_{50} value, calculated from a dose–response curve, represents the dose of a drug (in mg/kg) producing screen failure in 50% of the mice tested. After the screen test, cocaine or saline was administered and locomotor activity or anticonvulsant efficacy was assessed as described below.

2.3. Anticonvulsant efficacy

After the screen test, a convulsant dose of cocaine (75 mg/kg ip) was administered and the mice were placed in individual Plexiglas containers (14 × 25 × 36 cm high) for observation. Mice in these experiments were used only once to evaluate the anticonvulsant efficacy of drugs. The dose of cocaine was chosen to be close to its ED_{80}–ED_{90} values as determined from the literature (Witkin and Tortella, 1991). The presence or absence of convulsions was monitored for 30



DM; R=R'=CH_3
DX; R=CH_3, R'=H
AHN 1019c; R=H, R'=CH_3
AHN 1050; R=$CH_2CH=CH_2$, R'=CH_3
AHN 1053; R=$CH_2CH=C(CH_3)_2$, R'=CH_3
AHN 1047; R=CH_3, R'=$CH(CH_3)_2$
AHN 1048; R=CH_3, R'=CH_2CH_3
AHN 1069; R=H, R'=CH_2CH_3
AHN 1080; R=CH_2Ph, R'=CH_2CH_3

Fig. 1. Chemical structures of DM, DX, and analogs.

min following cocaine. Cocaine-induced convulsions were defined as loss of the righting response for at least 5 s and the occurrence of clonic limb movements. Tonus and death were rarely observed. Depression of movement with loss of righting response often preceded clonic episodes in cocaine-challenged mice although bouts of wild running were also prominent in the preseizure progression. Once seizures developed in cocaine-treated mice, loss of the righting response often persisted over the 30-min observation period.

2.4. Locomotor activity

Mice were pretreated with the test compound and were returned to their home cages. After 30 min, performance on the inverted screen was assessed after which the mice received either saline or cocaine (10 mg/kg ip) injections and were placed in separate locomotor activity chambers. Monitoring of locomotor activity was performed during 30 min in a 40-cm^3 Digiscan activity monitor equipped with infrared beams aimed at photoelectric detectors placed 2.6 cm apart along the perimeter capable of sensing movement at a height of up to 20 mm off the floor (Omnitech Electronics, Columbus, OH). Activity levels were recorded for 30 min immediately after saline or cocaine administration, by incrementing a counter with each interruption of two successive photoelectric detectors. Each drug, drug combination, or vehicle was evaluated in separate groups of at least eight mice each, and doses were evaluated in a mixed order.

2.5. Data analysis

The quantal data for both the anticonvulsant and behavioral toxicity tests were evaluated according to the methods described by Litchfield and Wilcoxon (1949), and ED_{50} and TD_{50} values with 95% confidence limits were derived from this analysis. For estimating the anticonvulsant potency of the drugs, the ED_{50} value was defined as the dose predicted to produce convulsions in 50% of the animals tested. Specific comparisons between control and drug treatments were performed with the Fisher's Exact Probability Test. Locomotor activity data were analyzed by a one-way ANOVA followed by the Dunnett's test for post hoc comparisons against the corresponding control group. For estimating the ED_{50} values with 95% confidence limits, the linear region of each dose–response curve was adjusted to a linear function. For the effects of the drugs on cocaine-evoked locomotor activity, the ED_{50} was defined as the dose of the drug that reduced cocaine-evoked locomotor activity to the intermediate value between the basal and cocaine evoked activity levels in the corresponding control, saline-pretreated groups. For the effects of the drugs on basal locomotor activity, the ED_{50} value was defined as the dose that reduced activity to 50% of the basal activity in the corresponding control, saline-treated group. Statistical probabilities of less than .05 were considered to be significant. Pearson correlation analysis of the relationship between potency and receptor affinity was performed using the ED_{50} values described above and the affinities of the DM analogs previously reported (Newman et al., 1996).

2.6. Drugs

All test compounds were dissolved in distilled water and were given subcutaneously 30 min before the saline or cocaine (ip) challenge. Drugs were injected in a volume of 0.01 ml/g. DX and the DM analogs (AHN 1019c, 1050, 1053, 1047, 1048, 1069, 1080) were synthesized as

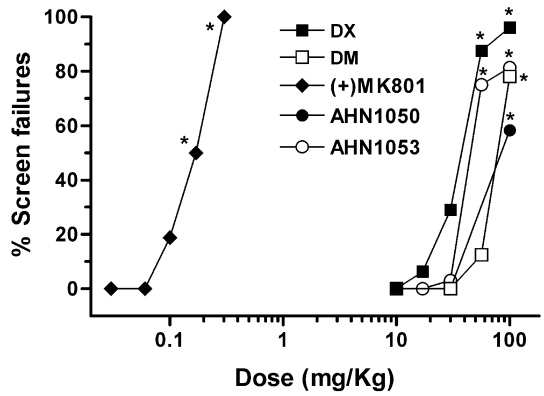


Fig. 2. Dose–response curves for the effects of DM analogs on the inverted screen test. Mice received subcutaneous injections of saline or the corresponding drug dose 30 min before testing in the inverted screen. Values are the % of animals failing the test (at least eight mice per group). None of the mice in the saline pretreated groups failed the test. Statistical comparisons were performed against a common saline control group of 16 mice. $*P<.05$ versus the saline pretreated group (Fisher's Exact Probability Test). Only the drugs displaying a significant effect are shown. For the highest inactive dose of the other compounds tested, see Table 1.

Table 1
Affinites of the DM analogs for sigma-1, sigma-2 and PCP binding sites and their potencies in behavioral and anticonvulsant tests

Compound	PCP affinity K_i (nM)	Sigma-1 affinity K_i (nM)	Sigma-2 affinity K_i (nM)	Screen failures	Cocaine seizures	Basal locomotor activity	Cocaine locomotor activity
DM	3500	419	2639	79 (65–96)	>100	44 (31–57)	30 (23–37)
AHN 1019c	487	1034	>10,000	>100	31 (12–82)	58 (48–68)	46 (22–70)
AHN 1050	1410	64	>1000	84 (53–134)	45 (30–70)	51 (43–59)	Not tested
AHN 1053	27,800	24	948	47 (40–56)	13 (9–19)	32 (25–38)	25 (15–36)
DX	460	559	1128	35 (27–45)	48 (24–95)	98 (69–126)	41 (34–47)
AHN 1047	11,500	2181	>1000	>30	13 (7–25)	28 (16–39)	16 (5–27)
AHN 1048	7300	461	>1000	>30	7 (6–9)	12 (8–16)	17 (12–21)
AHN 1069	1770	2078	>10,000	>30	17 (12–23)	15 (12–19)	7 (2–11)
AHN 1080	40,200	8	1050	>30	>30	>100	>100
NPC 16377				>100	Partial efficacy	63 (36–90)	8 (4–13)
(+)-MK-801				0.16 (0.10–0.24)	0.21 (0.16–0.28)	Increase	0.027 (0.01–0.04)

ED_{50} values (mg/kg) calculated from the dose–response curves. The 95% confidence interval is shown in parentheses. The affinity values are taken from a previous report (Newman et al., 1996). PCP affinity was obtained by displacement of [^{3}H]TCP binding. Sigma-1 affinity was assessed by the displacement of [^{3}H]SKF 10047 binding in the presence of MK-801. Sigma-2 binding was obtained by displacement of [^{3}H]DTG binding in the presence of (+)-SKF-10047 (see Newman et al., 1996) for details.

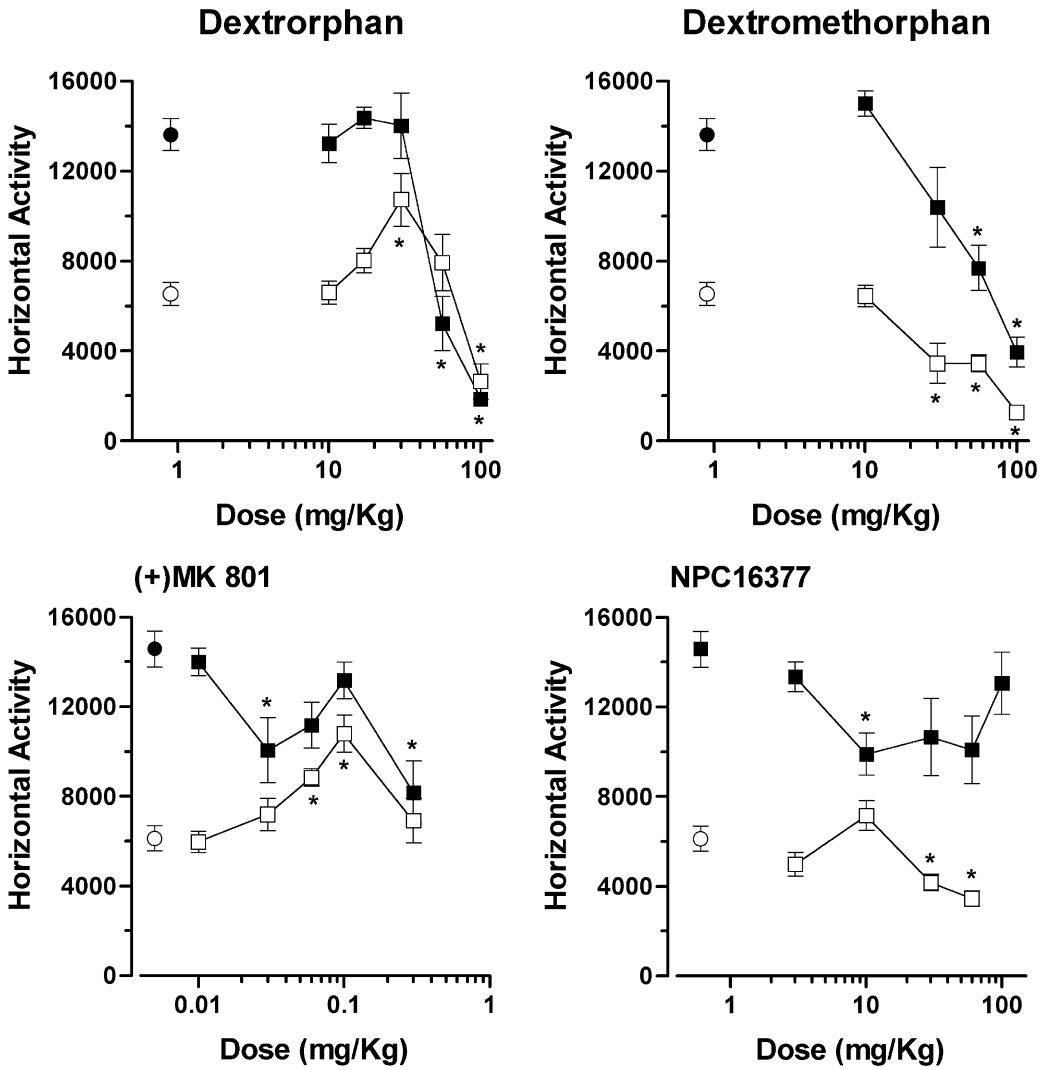


Fig. 3. Dose–response curves for the effects of DX, DM, (+)-MK-801, and NPC 16377 on basal (open symbols) and cocaine-induced (filled symbols) locomotor activity (photobeam interruptions). Mice received subcutaneous injections of saline (circles) or the corresponding drug dose (squares) 30 min before an intraperitoneal saline or cocaine (10 mg/kg) challenge. Immediately after the challenge, mice were placed in the locomotor chambers and activity was scored for 30 min. Values are mean ± S.E.M. of at least eight mice per group. * $P < .05$ versus the corresponding saline pretreated group (Dunnett's test).

described (Newman et al., 1996). Dizocilpine [(+)-MK-801] was obtained from Research Biochemicals, Int. (Natick, MA), DM from Sigma (St. Louis, MO) and (−)-Cocaine HCl was obtained from the National Institute on Drug Abuse (Rockville, MD). NPC 16377 was donated by the former Nova Pharmaceutical (Baltimore, MD).

3. Results

3.1. Behavioral toxicity

The chemical structures of DM, DX, and the DM analogs are shown in Fig. 1. Behavioral toxicity of the compounds

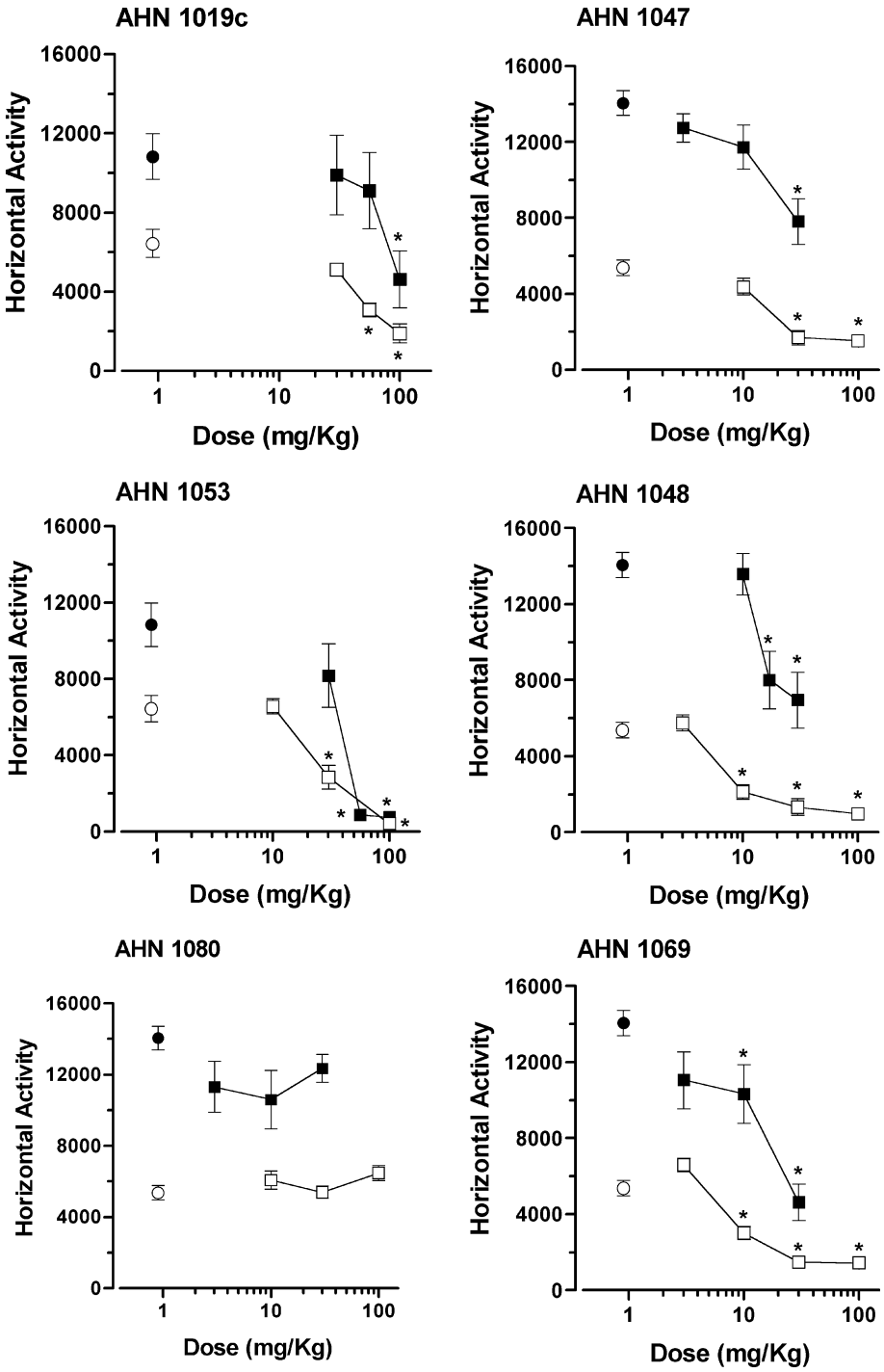


Fig. 4. Dose–response curves for the effects of the DM analogs on basal (open symbols) and cocaine-induced (filled symbols) locomotor activity. The injection regimen is as per Fig. 3. Values are mean ± S.E.M. of at least eight mice per group. * $P < .05$ versus the corresponding saline pretreated group (Dunnett's test). AHN 1050 was not tested against cocaine-evoked locomotor activity due to limitations in the availability of this compound.

was assessed in the inverted screen test and the data obtained are presented in Fig. 2. All the mice in the saline-treated, control groups rapidly climbed to the top of the wire mesh. In general, the DM analogs presented a better toxicity profile than the parent compound. Thus, only three of the compounds (AHN 1050, AHN 1053, and DX) showed significant behavioral impairing effects in the inverted screen test. The remainder of the DM analogs did not significantly increase the number of screen failures at the doses tested (see Table 1). While AHN 1050 and AHN 1053 impaired performance at doses that evoked a profound suppression of locomotor activity, the behavioral impairing effects of DX were observed at doses that increased locomotor activity (see below). The NMDA channel blocker, (+)-MK-801, also potently impaired performance in the inverted screen test at the same dose range that produced locomotor stimulating effects, while NPC 16377, the selective sigma ligand, did not induce any impairing effects at any of the doses tested.

Table 3
Protective indices of DM, analogs, and comparison compounds

Compound	Screen failures/ cocaine seizures	Basal activity/ cocaine seizures	Screen failures/ cocaine activity	Basal activity/ cocaine activity
DM	<0.80	<0.44	2.6	1.5
AHN 1019c	>3.2	1.8	>2.2	1.3
AHN 1050	1.9	1.1	–	–
AHN 1053	3.6	2.5	1.9	1.3
DX	0.73	2.0	0.85	2.4
AHN 1047	>2.3	2.2	>1.9	1.8
AHN 1048	>4.3	1.7	>1.8	0.70
AHN 1069	>1.8	0.88	>4.3	2.1
AHN 1080	–	–	–	–
NPC 16377	–	–	>12.5	7.9
(+)-MK-801	0.76	–	5.9	–

The PI is the ratio of the TD_{50} (basal locomotor activity-decreasing effect or screen failure—Table 1) to the ED_{50} to protect against cocaine-stimulated locomotion or cocaine-induced seizures (Table 1). A PI of greater than 1 is indicative of a favorable side-effect profile. No entries were made when calculation was not possible.

3.2. Locomotor activity

The effects of the compounds on locomotor activity are presented in Figs. 3 and 4, and the potencies are summarized in Table 1. The results of the ANOVA tests are presented in Table 2. Cocaine (10 mg/kg) stimulated locomotor activity in all of the saline pretreated control groups. The cocaine-induced increase in activity ranged from 168% to 262% of the basal values obtained in the corresponding saline pretreated groups.

DM inhibited cocaine-stimulated locomotor activity at doses which also reduced basal activity levels. However, significant blockade of cocaine-induced locomotor activity was observed at doses that did not significantly impair performance in the inverted screen test. Thus, the ED_{50} value calculated from the dose–response curves was significantly lower for blocking the cocaine stimulating effects than for inducing impairment in the screen test (Tables 1 and 3).

The DM analogs blocked the locomotor stimulant effects of cocaine at doses that inhibited basal locomotor activity, with the exception of AHN 1080 that had no effect on either cocaine-stimulated or basal locomotor activity and AHN 1050 that was not tested against cocaine stimulation (due to lack of supply of AHN 1050) but reduced basal activity levels. The ED_{50} values for inhibiting the locomotor stimulant effects of cocaine were not significantly different from the ED_{50} values for reducing basal activity, except for AHN 1069. All of the DM analogs were effective as blockers of cocaine-stimulated locomotor activity at doses which also decreased basal locomotor activity but that were devoid of behavioral impairing effects in the screen test (with the exception of DX, see also Table 3).

In contrast to the DM-based compounds, (+)-MK-801, increased basal locomotor activity at moderate doses and decreased activity at higher doses. DX, a DM derivative with significant affinity for the PCP binding site, also altered basal locomotor activity in a similar biphasic pattern. (+)-MK-801 blocked the locomotor stimulant effects of cocaine at the higher doses. However, a low dose of (+)-MK-801 was also able to significantly decrease the cocaine-evoked locomotor stimulation without altering basal activity levels.

As with DM and its derivatives, the selective sigma ligand NPC 16377 decreased basal locomotor activity. However, NPC 16377 differed from these compounds in that it only partially attenuated the stimulatory effects of cocaine.

Table 2
Results of the ANOVA tests performed on basal and cocaine-evoked locomotor activity

Variable	Basal locomotor	Cocaine-evoked locomotor
DX	$F(5,45)=9.683$ **	$F(5,46)=35.130$ **
DM	$F(4,38)=15.524$ **	$F(4,39)=19.602$ **
AHN 1019c	$F(3,28)=17.470$ **	$F(3,31)=3.057$ *
AHN 1053	$F(3,28)=33.826$ **	$F(3,39)=13.535$ **
AHN 1047	$F(3,36)=22.147$ **	$F(3,36)=8.837$ **
AHN 1048	$F(4,43)=29.196$ **	$F(3,36)=11.570$ **
AHN 1069	$F(4,37)=28.793$ **	$F(3,36)=13.783$ **
(+)-MK-801	$F(5,42)=7.243$ **	$F(4,35)=4.945$ **
NPC 16377	$F(4,35)=8.582$ **	$F(5,42)=2.564$ *

* $P<.05$.
** $P<.01$.

3.3. Cocaine-evoked seizures

The effect of the compounds on cocaine-induced seizures is shown in Fig. 5 and the potencies reported in Table 1. DM, at the doses tested, did not significantly protect against cocaine-induced seizures. However, a positive trend was observed at the highest dose tested (100 mg/kg, P=.07). All the DM analogs but one (AHN 1080) protected against

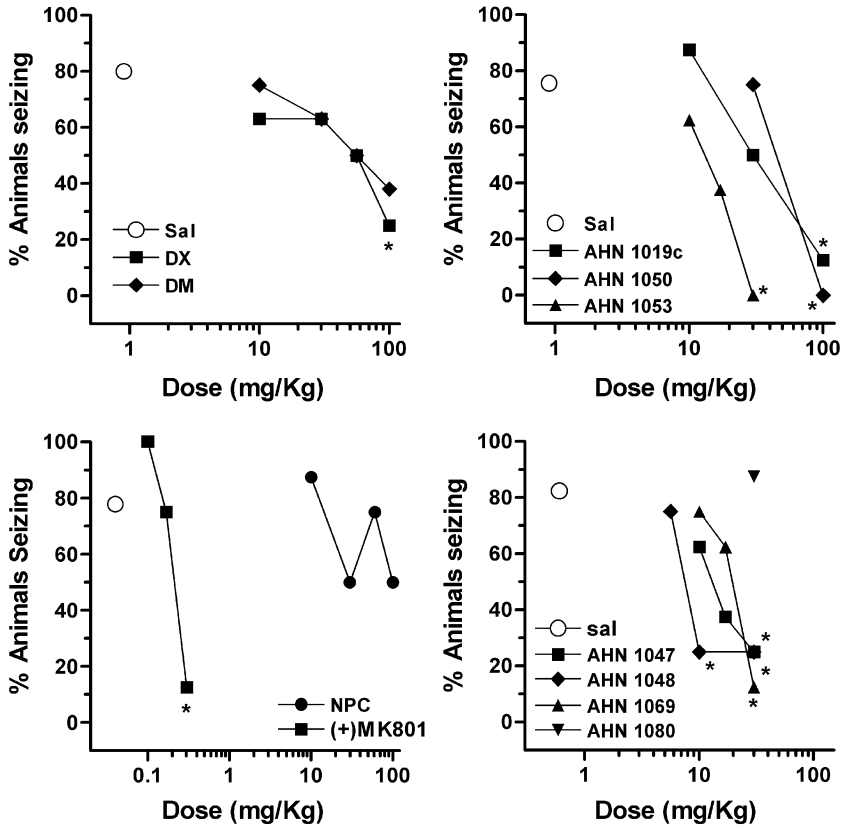


Fig. 5. Dose–response curves for the effects of the DM analogs on cocaine-induced seizures. Mice received subcutaneous injections of saline (open circles) or the corresponding drug dose (filled symbols) 30 min before a cocaine injection (75 mg/kg ip). Values are the % of animals showing seizures (at least eight mice per group). * $P < .05$ versus the saline pretreated group (Fisher's Exact Probability Test). Only the drugs displaying a significant effect are shown. Only one high dose of AHN 1080 was studied due to limited supplies.

cocaine-induced seizures with full efficacy (but only one high dose of this compound was studied due to the lack of compound availability). The selective sigma ligand NPC 16377 showed a trend to a partial protection. The NMDA receptor channel blocker (+)-MK-801 completely prevented cocaine-induced convulsions, but only at doses that impaired performance in the inverted screen test and increased locomotor activity. In general, the potencies of the DM analogs in preventing cocaine seizures were significantly lower than the potency to induce motor impairments in the inverted screen test, with the exception of AHN 1050 and DX (see Table 1).

3.4. Protective indices (PIs)

The separation between the doses that block behavioral or toxic effects of cocaine and the doses that have effects on their own was calculated (Table 3). PIs greater than 1 indicate that blockade occurs at doses lower than those having behavioral side effects. DM displayed PIs greater than 1 for blockade of cocaine-stimulated locomotion but less than 1 for blockade of cocaine-induced seizures. DX showed a better separation when basal activity was used as the side-effect measure rather than screen failures. MK-801 displayed a large separation between doses producing screen

Table 4
Correlation coefficients (Pearson's r) of the parameters presented in Table 1

	PCP affinity K_i (nM)	Sigma-1 affinity K_i (nM)	Cocaine seizures	Basal locomotor activity	Cocaine locomotor activity
Cocaine seizures	− .790 (.035)	− .095 (.839)	1	.873 (.010)	.576 (.231)
Basal locomotor activity	− .568 (.142)	− .195 (.644)	.873 (.010)	1	.836 (.019)
Cocaine locomotor activity	− .367 (.418)	− .324 (.479)	.576 (.231)	.836 (.019)	1

Only the DM structurally related compounds were included in the correlation [NPC 16377 and (+)-MK 801 were not considered]. The compound AHN 1080 was also not included due to a lack of effect on any of the dependent variables. The two-tailed P value is shown in parentheses.

failures and blockade of cocaine-stimulated activity. NPC 16377 had the highest PI values. Several of the DM analogs had PI values that were generally and consistently greater than unity (AHN 1019c, 1050, 1053, and 1047). Other DM analogs had PI values greater than unity only for specific measures.

3.5. Correlation analysis

The results of the correlation analysis are shown in Table 4. Only the structural analogs of DM were included in the analysis [NPC 16377 and (+)-MK-801 were not considered]. AHN 1080 was discarded because it did not have any observable effects on any of the parameters measured, suggesting that it might not have provided appropriate brain exposure. A significant negative correlation was observed between the affinity at the PCP site and the potency in inhibiting cocaine-induced seizures. No other significant correlation was found between any of the dependent variables and the affinity at either the PCP site of the NMDA receptor or the sigma-1 binding site. However, significant correlations were found between the dependent variables. Thus, the potencies to inhibit both cocaine-induced locomotor activity and seizures were significantly correlated with the ability of the compounds to inhibit basal locomotor activity.

4. Discussion

A series of DM analogs was evaluated for their abilities to block cocaine-induced seizures and locomotor activity. The data obtained show that these compounds are potent blockers of these effects of cocaine at doses that do not show any significant ataxic/sedative side effects as measured in the inverted screen test. The effects of these compounds against the behavioral and toxic effects of cocaine add to the data on their efficacy and favorable side-effect profile as neuroprotective agents and as anticonvulsants against other convulsant stimuli (Tortella et al., 1994, 1999). The efficacy of the DM analogs reported here is also notable given the general resistance of the convulsions induced by cocaine under the present conditions to a broad range of classical anticonvulsant compounds (Witkin et al., 1999).

In addition to their efficacy, the DM analogs displayed a generally favorable separation between doses that protected against effects of cocaine and doses that produced behavioral effects when given alone (PI); the separation being most striking when screen failures are used as the side-effect marker. The selective sigma ligand, NPC 16377, also had high PIs for blockade of locomotor activity but, in contrast to DM and its analogs, NPC 16377 was only partially effective against cocaine-evoked seizures in the present study. The PI greater than unity obtained for DX and (+)-MK-801 may be accounted for by two different reasons. First, these compounds increased locomotion at lower doses and decreased (DX) locomotion at higher doses. Therefore, if the ascending portion of the dose–effect function was used in computation of the PI for DX, the PI would be 1 or less, a finding consistent with the efficacy and behavioral side-effect profile of DX and (+)-MK-801 to be within roughly similar dose ranges (Ginski and Witkin, 1994; Geter-Douglass and Witkin, 1999). The large PI for (+)-MK-801 as a blocker of cocaine-stimulated activity is due to the finding that a low dose was capable of blocking this effect. Bespalov et al. (2000) also reported that a low dose of (+)-MK-801 (0.03 mg/kg) attenuated the conditioned locomotor stimulant effects of cocaine in rats and higher doses have been shown to slightly reduce cocaine hyperactivity in mice (Uzbay et al., 2000) as reported here at 0.3 mg/kg. The blockade of cocaine-induced locomotor activity is not universally observed (Wolf et al., 1994). The narrow dose window for low-dose blockade and the induction of behavioral stimulation at somewhat higher doses of MK-801 may be factors accounting for the discrepancy in the literature. Nonetheless, the present findings in conjunction with the data from the AMPA antagonist, NBQX (findings in Witkin, 1993), demonstrate that glutamatergic stimulation by cocaine plays a role in the stimulant effects of cocaine.

In human studies, adverse effects following administration of high-affinity NMDA ion channel blockers are notable. Psychomimetic effects that resemble schizophrenia including hallucinations, paranoia, emotional withdrawal, and motor retardation have been observed following administration of PCP and related dissociative anesthetics including DX (Snyder, 1980; Krystal et al., 1994; Albers et al., 1995; Muir and Lees, 1995). Similar effects have been reported with DM (Dodds, 1967). Furthermore, PCP is an abused substance and DM has likewise been reported to have dependence-producing effects (Fleming, 1986). During clinical trials for the treatment of epilepsy, dizocilpine produced poor therapeutic efficacy in some patients, while in others psychomimetic effects were observed at therapeutic doses (Leppik et al., 1988). The analogs of DM studied here produce effects that distinguish them from these PCP-like NMDA receptor antagonists. Although they produced failures on the inverted screen test like other PCP-like blockers, they did not stimulate locomotor activity, an effect common to PCP-like antagonists (Ginski and Witkin, 1994) as also reported with (+)-MK-801 and DX in the present experiment. Indeed, the analogs of DM were designed (Newman et al., 1996) to overcome the propensity for metabolic conversion to the PCP-like agent DX (Barnhart, 1980; Tortella et al., 1994). Thus, it is predicted that these compounds may produce less liability for psychomimetic effects in humans. It is possible that the generally lower affinity of the DM analogs studied may contribute to their more favorable side-effect profile as has been noted with lower-affinity NMDA receptor ligands (Geter-Douglass and Witkin, 1999). The role of the putative novel pharmacological actions of the DM analogs (as discussed

below) may also be important in defining the side-effect profile of these compounds.

DM has been shown to have weak or no effects upon cocaine-evoked seizures, depending on the cocaine dose and administration route employed. Thus, it has been reported that DM is able to partially inhibit seizure incidence (Barat and Abdel-Rahman, 1997) or increase the seizure threshold (Karler and Calder, 1992) induced by intravenous cocaine in rats. However, a previous study using intraperitoneal cocaine administration in mice has shown no effect of DM (Witkin and Tortella, 1991). In the present work, we did not observe a significant protection of DM against cocaine-evoked seizures, although a trend was clearly apparent at high DM doses, which also produced significant sedative/ataxic effects. In contrast, the DM analogs prevented cocaine-evoked seizures with full efficacy and with potencies several times lower than the doses required for inducing significant motor impairing effects. The present results strongly argue against an involvement of the PCP binding site in the ability of these DM analogs to prevent cocaine-evoked seizures. Although the PCP-like compounds dizocilpine and DX also fully protected against cocaine seizures, they did so only at doses that induced a marked motor impairment. Moreover, the anticonvulsant potencies of this series of compounds were, in fact, negatively correlated with their K_i values for displacing [^{3}H]TCP binding, suggesting that the affinity for the PCP binding site of the NMDA receptor not only does not mediate the anticonvulsant effects of these compounds but, in fact, impairs their ability to block cocaine seizures. In contrast, using structurally diverse NMDA receptor blockers, a positive association between anticonvulsant potency and affinity was previously observed for ligands blocking the PCP site (r=.80, n=9, P<.05) as well as for those blocking the glutamate recognition site (r=.79, n=7, P<.05) (Witkin et al., 1999). Moreover, functional antagonists at other sites on the NMDA receptor ion complex (glycine, polyamine) also prevent these anticonvulsant resistant cocaine seizures (Witkin et al., 1999; Brackett et al., 2000). Thus, although functional blockade of the NMDA receptor clearly is able to prevent cocaine-induced seizures (Witkin and Tortella, 1991; Witkin et al., 1999), it does not appear to be involved in the anticonvulsant actions of these compounds at least in so far as cocaine is the convulsant stimulus.

As observed with anticonvulsant effects, the effects of these compounds on cocaine-stimulated locomotor activity do not seem to be mediated through their blockade of the PCP binding site of the NMDA receptor since their potencies to prevent cocaine locomotor stimulant effects were not correlated with their affinities for the PCP site. In addition, the effects of PCP-like ligands, like DX or MK-801, on basal locomotor activity display a characteristic biphasic dose–response curve, with lower doses increasing locomotor activity. This was not the case with any of the DM derivatives, which decreased basal locomotor activity in a monophasic fashion. Even AHN 1019c, which had a comparable affinity to that of DX at the PCP binding site, did not stimulate activity. This fact in addition to others cited here point to non-NMDA mechanisms in the behavioral pharmacology of this structural series.

There is increasing evidence that sigma ligands can modulate some of the behavioral actions of cocaine. It has been reported by different laboratories that compounds with affinity for the sigma receptors are able to inhibit the locomotor stimulant effects of cocaine (Menkel et al., 1991; Witkin et al., 1993; Hascoet et al., 1995; Maj et al., 1996; McCracken et al., 1999a,b). In addition, there is evidence that compounds acting at sigma receptors protect against cocaine-induced seizures (McCracken et al., 1999a), although in some studies only with partial efficacy (Witkin et al., 1993). In the present work, the selective sigma compound NPC 16377 inhibited cocaine-stimulated locomotor activity but did not significantly protect against cocaine-induced seizures, although it showed a trend towards a partial efficacy as previously reported (Witkin et al., 1993). The higher dose of cocaine used in the present experiment (75 vs. 60 mg/kg in Witkin et al., 1993) may have been a contributing factor as reported with other anticonvulsant agents (Witkin and Tortella, 1991). DM has moderate (K_i=420 nM) affinity for the sigma-1 receptor, and some of the DM analogs are potent sigma-1 ligands with low nM affinities. However, the anticonvulsant potencies of this series of compounds did not significantly correlate with their affinities for the sigma-1 binding site. In fact, the most potent and selective ligand for sigma-1 receptors, AHN 1-080, was the only DM analog without any protective action against cocaine-induced seizures (noting again, however, that only a single dose could be studied due to lack of compound availability). None of the compounds exhibited high affinities for sigma-2 receptors. Thus, the present results do not support the involvement of sigma-1 or sigma-2 receptors in the effects of these DM analogs on cocaine-evoked seizures and locomotor stimulation. Similarly, a lack of relationship between sigma receptor affinity and potency to protect against cocaine convulsions has been reported with other compounds that share both NMDA and sigma receptor activities (Witkin et al., 1999).

Interestingly, the potency in inhibiting basal locomotor activity was significantly correlated with both the potency to inhibit cocaine-evoked seizures and locomotor activity, suggesting that a single mechanism might be responsible for these behavioral effects. It could be argued that with compounds with low affinities at either the PCP or the sigma site, a higher affinity at the other site could account for its behavioral effects, thus explaining the apparent lack of significant correlation with either single site. In other words, affinity at both sites could contribute to their overall pharmacological profile. However, this interpretation is unlikely because compounds displaying low affinities at both sites (e.g. AHN 1047 and AHN 1069) still had potent and efficacious behavioral effects. This latter point raises again the possible involvement of non-sigma, non-NMDA

receptor mechanisms in the effects of the current series of DM analogs. It is known that DM binds to at least two high-affinity sites in brain. While one is the common sigma-1 site, the other high-affinity site is insensitive to (+)-3-PPP, DTG, (+)-pentazocine or modulation by ropizine, suggesting a specific DM binding site, different from the sigma-1 receptor recognized by the sigma ligands (Klein and Musacchio, 1992). However, it is not known at present what contribution, if any, this DM specific binding site may play.

Finally, another factor to consider is the possibility of a pharmacokinetic interaction between the DM derivatives and cocaine. There is no information currently available on the metabolism of these compounds. The possibility exists that the behavioral actions of these compounds are mediated by some of their metabolic products. DM itself is a substrate for certain enzymes in the cytochrome *P*-450 family (Gorski et al., 1994), which are also involved in cocaine metabolism (Pellinen et al., 1994), raising the possibility of an interaction at the metabolic level. Thus, they might alter cocaine metabolism and decrease the concentration of cocaine reaching the brain. Finally, they also could alter the production of the several degradation products of cocaine. There is evidence that some cocaine metabolites could be involved in its toxic effects (Mets and Virag, 1995). To test this possibility, a full pharmacokinetic study assessing the temporal course of both cocaine and the DM derivatives metabolites should be implemented.

DM has been shown to alter intravenous cocaine self-administration in the rat (Kim et al., 1997; Pulvirenti et al., 1997). In addition, there is evidence that DM could share some discriminative stimulus effects with cocaine (Gauvin et al., 1998). These actions of DM have been attributed to its weak NMDA antagonist activity. However, the results reported here suggest that DM and related compounds can block some of the behavioral actions of cocaine by a mechanism not related to modulation of NMDA or sigma receptors. Several DM derivatives have previously been shown to display potent anticonvulsant activity in the rat maximal electroshock seizure model (Tortella et al., 1994). Since these compounds are predicted to have a reduced probability of PCP-like side effects in humans as has been documented with other NMDA receptor antagonists, this chemical class could be potentially useful in blocking some of the behavioral effects of cocaine in humans. Elucidation of the mechanism underlying behavioral and protective effects of these compounds could, in addition, shed light on the neurochemical underpinnings of the stimulant and convulsant effects of cocaine.

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