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The anticonvulsant and neuroprotective activity of dextromethorphan (DM, [+]3-methyl-17-methylmorphinan) may be, in part, due to its metabolism to the PCP-like compound dextrorphan (DX). We evaluated the anticonvulsant activity and neurological impairing effects in rats of three novel analogs of DM which, based upon their 3-position substituents, would either not be expected to be metabolized to DX, or might do so at a reduced rate. The DM analogs were determined to be more potent and more efficacious than DM against maximal electroshock (MES) convulsions; two of the analogs, namely AHN1-036 ([+]-3-ethoxy-17-methylmorphinan) and AHN1-037 ([+]-3-(2-propoxy)-17-methylmorphinan), were equipotent to DX. AHN1-036 and AHN1-037 exhibited a duration of action (1-2 hrs) slightly longer than DX (0.5-1 hr) and similar to DM (2-4 hr). The anticonvulsant effect of AHN649 persisted 4-6 hrs. Against flurothyl convulsions DM was proconvulsant, DX was anticonvulsant, and the DM analogs were inactive. In contrast, N-methyl-D-Aspartate (NMDA) convulsions were antagonized by pretreatment with DM and the DM analogs, albeit with a potency approximately 10 times less than that of DX. Results of rotarod performance testing further distinguished the analogs from DM, DX or the anticonvulsant drug diazepam (DZ). No behavioral impairment was observed at the highest doses tested of each of the DM analogs resulting in protective indices (i.e. rotarod TD₅₀/MES anticonvulsant ED₅₀) greatly exceeding DM, DX or clinical anticonvulsant drugs. The results of this study establish these 3-substituted DM analogs as novel anticonvulsants exhibiting improved potency, efficacy, duration and side-effect profiles.

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Novel Anticonvulsant Analogs of Dextromethorphan: Improved Efficacy, Potency, Duration and Side-Effect Profile¹

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ABSTRACT

The anticonvulsant and neuroprotective activity of dextromethorphan (DM, [(+)-3-methyl-17-methylmorphinan]) may be, in part, due to its metabolism to the phencyclidine hydrochloride-like compound dextrorphan (DX). We evaluated the anticonvulsant activity and neurological impairing effects in rats of three novel analogs of DM which, based upon their position-3 substituents, would either not be expected to be metabolized to DX or might do so at a reduced rate. The DM analogs were determined to be more potent and more efficacious than DM against maximal electroshock convulsions; two of the analogs, namely [(+)-3-ethoxy-17-methylmorphinan] and [(+)-3-(2-propoxy)-17-methylmorphinan], were equipotent to DX. [(+)-3-Ethoxy-17-methylmorphinan] and [(+)-3-(2-propoxy)-17-methylmorphinan] exhibited a duration of action (1–2 hr) slightly longer than DX (0.5–1 hr) and similar to DM (2–4 hr). The anticonvulsant effect of [(+)-

3-amino-17-methylmorphinan] persisted 4–6 hr. Against flurothyl convulsions DM was proconvulsant, DX was anticonvulsant and the DM analogs were inactive. In contrast, N-methyl-D-aspartate convulsions were antagonized by i.c.v. pretreatment with DM and the DM analogs, albeit with a potency approximately 10 times less than that of DX. Results of rotarod performance testing further distinguished the analogs from DM, DX or the anticonvulsant drug diazepam. No behavioral impairment was observed at the highest doses tested of each of the DM analogs, resulting in protective indices (i.e., rotarod TD_{50} /maximal electroshock anticonvulsant ED_{50}) greatly exceeding DM, DX or clinical anticonvulsant drugs. The results of this study establish these 3-substituted DM analogs as novel anticonvulsants exhibiting improved potency, efficacy, duration and side-effect profiles.

DM, the active pharmaceutical in many over-the-counter cough suppressants, exhibits a broad-spectrum anticonvulsant profile (Tortella *et al.*, 1989b; Kuko and Wada, 1989; Takazawa *et al.*, 1990) as well as neuroprotective properties in experimental models of neuronal injury (Steinberg *et al.*, 1988; George *et al.*, 1988; Tortella *et al.*, 1989a). It has been speculated that the mechanism of action of these effects may be related to the weak NMDA antagonist properties of DM (Church *et al.*, 1985; Aram *et al.*, 1989; Tortella *et al.*, 1989b) or to binding to distinct high-affinity DM/ σ recognition sites in the brain (Musacchio *et al.*, 1989; Tortella *et al.*, 1989b; Walker *et al.*, 1990; Zhou and Musacchio, 1991). However, it is well known that DM is metabolized to the phenolic PCP-like compound DX (Kamm *et*

al., 1967; Barnhart, 1980), which is a more potent anticonvulsant (Tortella *et al.*, 1988a; Wong *et al.*, 1988; Aram *et al.*, 1989; Chapman and Meldrum, 1989), neuroprotectant (Choi, 1987; Choi *et al.*, 1987; Monyer and Choi, 1988; Steinberg *et al.*, 1988, 1989) and calcium channel antagonist (Carpenter *et al.*, 1988) in many of the same model systems. This metabolite may be responsible for all, or at least some, of the pharmacological properties of the parent drug.

Although DM and DX have many actions in common (Leander, 1989), several distinct differences exist between their binding characteristics and *in vivo* pharmacology. Most notably, 1) DM exhibits high binding potency to distinct DM recognition sites (12–57 nM; Craviso and Musacchio, 1983; Klein and Musacchio, 1989) and σ binding sites classified as σ -1 (121 nM; Walker *et al.*, 1990; Rothman *et al.*, 1991), while demonstrating relatively low affinity for sites labeled by DX (321 nM, Franklin and Murray, 1992) or PCP (513 or 2500 nM; Klein and Musacchio, 1989; Murray and Leid, 1984, respectively). In contrast, DX exhibits high affinity for specific DX or PCP sites in the rat brain (39.5 and 23 nM, respectively

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¹ In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4–3, AR 360–5).

ABBREVIATIONS: DM, dextromethorphan; NMDA, N-methyl-D-aspartate; PCP, phencyclidine hydrochloride; DX, dextrorphan; AHN649, [(+)-3-amino-17-methylmorphinan]; AHN1-036, [(+)-3-ethoxy-17-methylmorphinan]; AHN1-037, [(+)-3-(2-propoxy)-17-methylmorphinan]; MES, maximal electroshock; PI, protective index; DZ, diazepam.

(Franklin and Murray, 1992), while exhibiting low to moderate affinity for DM and σ -1 sites (310 and 202 nM, respectively) (Klein and Musacchio, 1989; Walker et al., 1990); 2) the behavioral profiles (Tortella et al., 1989b; Szekely et al., 1991) of these drugs are distinct; and 3) similar to what has been observed in binding studies (Musacchio et al., 1989), the classical anticonvulsant drug phenytoin potentiates the anticonvulsant activity of DM (Tortella and Musacchio, 1986), but not DX (Tortella et al., 1988a). Furthermore, it has been shown recently that DM can be differentiated from PCP ligands like DX on the basis of their ability to attenuate NMDA- and K^+ -evoked increases in cytosolic Ca^{++} concentrations (Church et al., 1991).

Despite these differences, the fact remains that DM will be > 90% metabolized to DX in humans. Because it would be predicted that DM doses higher than the recommended antitussive dose (15–30 mg/kg) would be required, the potential undesirable PCP-like qualities of a DX metabolite would severely hamper the future development of this drug as a useful therapeutic entity for treatment of epilepsy, stroke or central nervous system trauma. Indeed, case reports of toxicity in children (Pender and Parks, 1991) and psychotomimetic reactions (Dodds, 1967; Fleming, 1986) associated with high-dose DM ingestion are likely attributable to this metabolite, as is the reported abuse potential in adolescent youths (Rammer et al., 1988; Pender and Parks, 1991).

We have explored recently the possibility of preparing DM analogs which are modified in position-3 of the morphinan ring system, with the intention of developing compounds that would retain anticonvulsant and neuroprotectant activity without *in vivo* conversion to DX (Newman et al., 1992). In our first series of compounds, three analogs including the aniline (AHN649), the *O*-ethylether (AHN1-036) and the *O*-isopropyl ether (AHN1-037) derivatives of DM (fig. 1) displayed anticonvulsant potency in rats which was equal to or greater than DM or DX (Newman et al., 1992). The purpose of the present study was to 1) evaluate further the pharmacological properties of the respective dose-response curves of these novel analogs for seizure protection in the MES test; 2) extend these findings to other experimental models of convulsant activity; 3) test for anticonvulsant activity after i.c.v. administration in which metabolism to DX is unlikely; and 4) use a rotorod assessment of

neurological impairment to determine the PI (Loscher and Nolting, 1991) for each of the respective compounds.

Methods

Animals. For all the seizure experiments male Sprague-Dawley rats were obtained from Zivic-Miller (Pittsburgh, PA). At delivery the animals were housed individually in a humidity and temperature controlled environment on a 12-hr light cycle beginning at 6:00 A.M. The rats were received at a weight range of 100 to 150 g and permitted food and water *ad libitum* during their acclimation period which lasted at least 7 to 10 days before testing.

Convulsant models. The anticonvulsant potency and efficacy of DM, DX, DZ and three of the DM analogs was determined in the MES test. For these experiments rats ($n = 10$ per group) weighing 175 to 225 g were used. Details of this supramaximal convulsion model of seizure spread and quantitation of anticonvulsant potency has been described elsewhere (Tortella et al., 1986). Briefly, electrical stimuli were applied auricularly *via* small alligator clip electrodes by using fixed supramaximal current stimulation (150 mA, 2 sec). The presence or absence of complete tonic hindlimb extension was measured. Anticonvulsant ED_{50} values and 95% CL were calculated from quantal dose-effect curves by using the method of Litchfield and Wilcoxon (1949) and the computer programs of Tallarida and Murray (1987).

For convulsant thresholds each of the drugs were tested for their ability to influence the onset to clonic convulsions induced by the volatile convulsant flurothyl (Adler, 1975; Tortella et al., 1986). In this model flurothyl is infused as a 10% solution in 95% ethanol (v/v) to groups of rats ($n = 8$ per group) weighing 225 to 275 g and placed individually in sealed test chambers. The seizure threshold was defined as the time interval between the start of the flurothyl infusion and the onset to a clonic convulsion. In the case of the DM analogs AHN1-036 and AHN1-037, where the amount of drug available for testing was limited, only a single dose equal to 2 times their respective MES anticonvulsant ED_{50} was tested.

For NMDA convulsions, rats weighing 200 to 250 g were initially anesthetized with halothane and implanted with an i.c.v. cannula aimed at the right lateral ventricle. Three to 5 days postsurgery the animals were divided into groups ($n = 6$ per group) for testing. Previous dose-response and pharmacological experiments from our laboratory have established 12.5 nM NMDA as a nonlethal, suprathreshold dose for inducing clonic "popcorn" convulsions in the rat *via* a direct interaction with the NMDA receptor complex (Robles et al., 1991). Two indices of convulsive activity were measured: the latency to onset to NMDA convulsions and the number of rats per group exhibiting convulsive behavior (*i.e.*, the percentage responding).

Rotarod performance. Rotarod experiments were carried out by using male Sprague-Dawley rats (260–370 g) obtained from Taconic Farms (Germantown, NY). The animals were trained to remain for 60 sec on a rotarod (model RRF, Omnitech Electronics, Columbus, OH) rotating at 6 revolutions/sec. Falling off the rotarod before 60 sec resulted in a 3 mA, 3 sec electric footshock. After testing to establish that the behavior was still intact, the subjects were treated with either saline or the test compound. Untreated animals were able to remain on the rod for several minutes. A positive score was given during testing if the rat failed to remain on the rotarod for 60 sec. The TD_{50} values and 95% CL for neurologic impairment were calculated from the quantal dose-effect curves as described above. The rotarod experiments were first conducted with groups of three or four rats per dose. If there was no effect (0% failures) at a particular dose level, no further testing with that dose was done. If, however, there was an effect then additional rats were run such that $n = 6$ was completed for that dose. The only exception to this design was for the AHN compounds where six rats were run at the highest doses reported, but no further increments were attempted due to the limited availability of these novel compounds.

Calculation of PI values and statistics. PI values were calculated by dividing the respective rotarod TD_{50} values by the MES anticonvulsant ED_{50} values established for each compound. Statistical analysis of

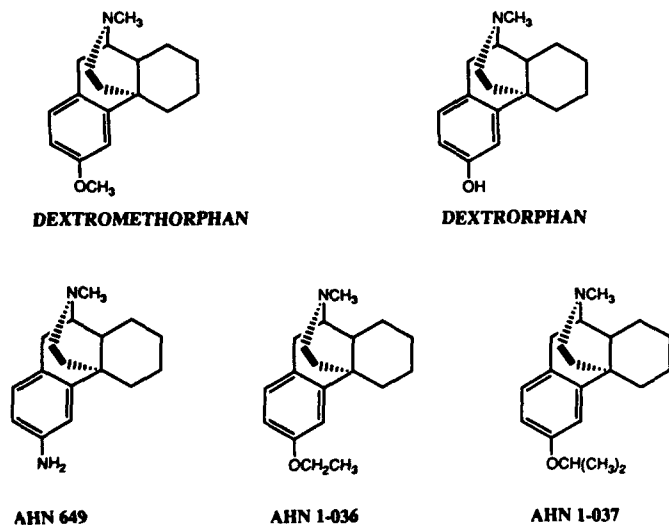


Fig. 1. Chemical structures of (+)-3-substituted morphinan analogs.

the dose-response curves for regression and parallelism was accomplished by using procedures 5 and 6 from Tallarida and Murray (1987). Flurothyl seizure thresholds were compared by using the Mann-Whitney *U* test (significance level, $P < .05$). The latency to NMDA convulsions was compared by using a one-way analysis of variance and a Student's *t* test (significance level, $P < .05$).

General protocol. In order to determine the time course of the anticonvulsant activity, compounds were tested at various times post-injection by using doses ($n = 6$ per dose) approximately equal to 2 times their respective MES anticonvulsant ED_{50} values. These experiments (fig. 3) determined the latency to peak anticonvulsant effect for DM, DX and the respective analogs to be 30 min after s.c. injection. Therefore, the pretreatment time for the MES, flurothyl and rotorod dose-response experiments was 30 min and the route of drug administration was s.c. (in a 1 or 2 ml/kg volume). For the i.c.v. NMDA experiments, pretreatment times were empirically set at 15 min. All i.c.v. injections were given as a rapid bolus (1-2 sec) in a 5- μ l volume followed by a 4- μ l vehicle flush. Throughout the study each rat was used only once and all rats were drug and seizure naive before testing. All experiments were carried out between 8:30 A.M. and 1:00 P.M. at an ambient temperature of 23-25°C.

Drugs. All drug solutions were made fresh by using distilled deionized water or saline. When necessary (i.e., with the highest doses of DM or AHN649) gentle heating was applied to enhance solubility. DM was obtained from Sigma Chemical Co. (St. Louis, MO). NMDA was obtained from Research Biochemicals Inc. (Natick, MA). DX and the DM analogs were synthesized as described previously (Newman *et al.*, 1992).

Results

The effect of the various compounds on MES convulsions is shown in figure 2, top. All the compounds tested were anticonvulsant, blocking the expression of tonic hindlimb extension in a dose-dependent manner. The dose-response curves for DM, DX and the respective DM analogs were linear (correlation coefficients were DM = [0.96], DX = [0.85], AHN649 = [0.95], AHN1-036 = [0.99] and AHN1-037 = [0.99]). Statistical comparison of these functions indicated that although the dose-response curves for DX and AHN-649 were parallel to DM, those produced by AHN1-036 and AHN1-037 were significantly different ($P < .05$ and $P < .01$, respectively). With the exception of DM, all the compounds were highly efficacious. In the case of DM, the highest dose tested (70 mg/kg) resulted in seizure protection in only 70% of the rats tested.

The anticonvulsant ED_{50} values are shown in table 1. The rank order of potency for the respective anticonvulsant drugs was DZ = AHN1-037 = DX = AHN1-036 > AHN649 = DM. At the highest doses tested rats given DZ appeared sedated whereas DX-treated rats exhibited mild ataxia. Rats given DM or the DM analogs appeared behaviorally normal at these doses.

Time course experiments showed peak anticonvulsant activity occurring within 30 min for all the compounds tested (fig. 3). Further analysis determined that the aniline analog, AHN649, retained at least 50% activity for as long as 4-hr postinjection. DM was also relatively long acting, producing seizure protection in 50% of the rats 2-hr postinjection. DX was the shortest acting anticonvulsant producing a marginal effect (20% protected) at 1-hr postinjection.

DM, DX and the analogs were tested for their ability to alter convulsant thresholds to flurothyl. Over the duration of the study control seizure thresholds matched to the various experimental groups ranged from 364 ± 9 to 402 ± 19 sec. Only DX was anticonvulsant in this seizure model, dose-dependently increasing the seizure threshold to $137 \pm 5\%$ of control (from

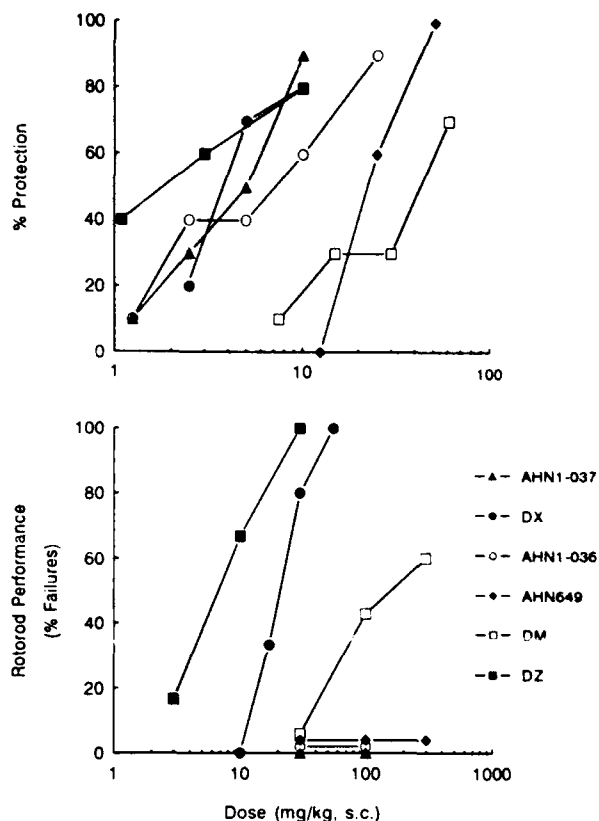


Fig. 2. Top, MES anticonvulsant dose-response curves. % Protection, the percentage of rats per group protected from MES-induced tonic hindlimb extension. Bottom, rotorod performance dose-response curves. % Failures, the percentage of rats per group failing to remain on the rotorod for 60 sec.

TABLE 1
MES anticonvulsant ED_{50} values, rotorod TD_{50} values (95% confidence intervals) and calculated PI values

Treatment	MES ^a	Rotorod ^b	PI
DZ	1.7 (0.6-5)	6.4 (3-15)	3.8
DM	37.8 (20-72)	229 (55-950)	6.0
DX	4.3 (3-7)	20.6 (14-31)	4.8
AHN649	21.4 (15-30) ^b	>300	>14.0
AHN1-036	5.6 (3-11) ^b	>100	>17.9
AHN1-037	3.9 (2-7) ^b	>100	>25.6

^a Values are expressed as milligrams per kilogram.

^b From Newman *et al.* (1992).

366 ± 9 to 500 ± 19 sec) at the highest dose tested (50 mg/kg). In contrast, DM, which at low doses (12.5 and 25 mg/kg) failed to influence the convulsant threshold to flurothyl, was proconvulsant at higher doses (50 and 75 mg/kg), significantly lowering the seizure threshold to $83 \pm 3\%$ of control (from 402 ± 19 to 333 ± 14 sec) at the highest dose tested (75 mg/kg, or 2 times its MES anticonvulsant dose). The DM analog AHN649 was inactive even at doses equivalent to 2 to 4 times its MES anticonvulsant ED_{50} . When tested at 2 times their MES anticonvulsant ED_{50} dose, AHN1-036 and AHN1-037 had no effect on the seizure threshold to flurothyl-induced convulsions. These results are summarized in table 2.

The effect of i.c.v. administered DM, DX and the respective DM analogs to antagonize convulsive activity induced by NMDA is demonstrated in figures 4 and 5. Administration of 12.5 nM NMDA directly into the brain of control, vehicle-

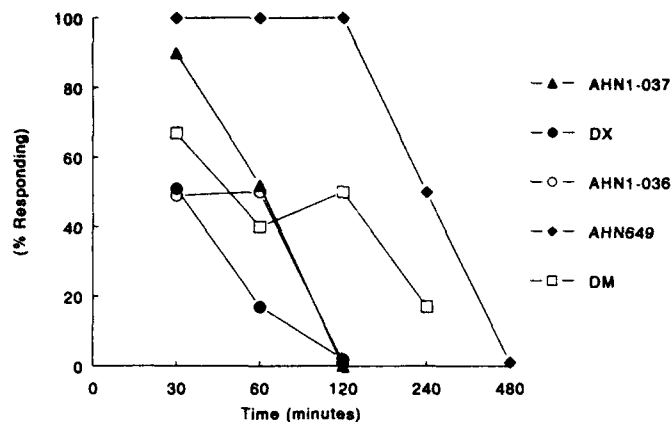


Fig. 3. Time course of drug-induced anticonvulsant activity. % Protection, the percentage of rats per group protected from MES-induced tonic hindlimb extension. Doses used are the same as defined in table 2 representing approximately 2 times the MES anticonvulsant ED_{50} dose for the respective compounds.

TABLE 2
Effect of DM, DX and the DM analogs on flurothyl seizure thresholds

Treatment	Dose	Seizure Threshold (% of control \pm S.E.) ^a
	mg/kg	%
DM	12.5	97 \pm 5
	25	98 \pm 5
	50	86 \pm 3*
	75 ^b	83 \pm 3*
DX	10 ^b	115%*
	25	122 \pm 5*
	50	137 \pm 5*
AHN649	50 ^b	101 \pm 4
	100	97 \pm 3
AHN1-036	12 ^b	98 \pm 4
AHN1-037	10 ^b	98 \pm 4

^a Because several control groups were used, the results have been normalized to 100% of the respective control seizure thresholds established for each group.

^b Doses representing approximately 2x the respective MES anticonvulsant ED_{50} for each compound.

* $P < .05$, Mann-Whitney U Test.

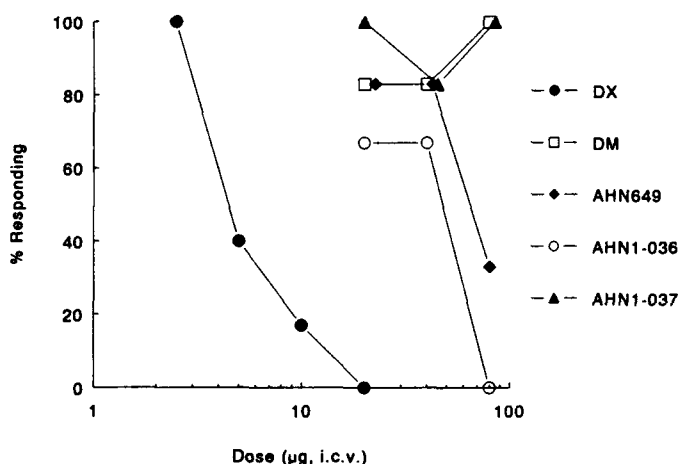


Fig. 4. Dose-dependent antagonism of NMDA-induced clonic popcorn convulsions. % Responding, the percentage of animals per group responding to NMDA with a clonic convulsion.

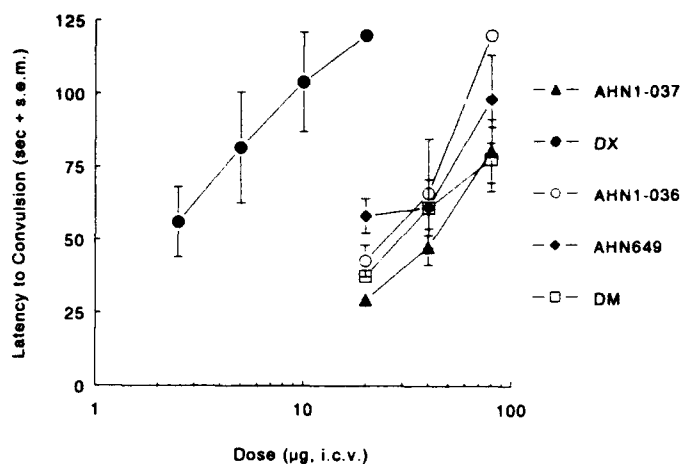


Fig. 5. Dose-dependent increase in the mean latency to NMDA-induced convulsions. Latency is determined from the initiation of the injection to the onset of behavioral convulsion. Each of the respective data points were analyzed to be significantly different from the control latency ($P < .05$, at least).

treated rats consistently resulted in a 100% incidence of severe, clonic (i.e., popcorn-like) convulsant activity. In these animals, the mean latency to the NMDA-induced convulsion was 16.7 ± 0.3 sec. Only DX, AHN649 and AHN1-037 dose-dependently antagonized the incidence of popcorn convulsions (fig. 4). However, pretreatment with each of the respective compounds resulted in a dose-related increase in the latency to the NMDA-induced convulsions (fig. 5). As can be seen from figures 4 and 5, dextrophan was approximately 10 times more potent than either DM or the DM analogs as an antagonist of the NMDA convulsion.

Figure 2, bottom describes the results of the rotarod performance studies. Of the drugs causing neurological impairment, DZ and DX were the most potent whereas DM produced marginal impairment in rotarod activity at doses as high as 300 mg/kg. None of the analogs impaired rotarod performance at doses as high as 100 to 300 mg/kg. The rotarod ED_{50} values are summarized in table 1.

By using the respective MES anticonvulsant ED_{50} and rotarod ED_{50} values, the PI values were calculated for each compound (table 1). The PI values for DX (4.8) and DZ (3.8) were similar. The PI for DM was slightly greater (6.0). Because even the highest doses of AHN649, AHN1-036 and AHN1-037 failed to impair rotarod performance, their respective PI values were determined to be, at a minimum, greater than 14, 17.9 and 25.6, respectively.

Discussion

Recognizing that a unique central nervous system pharmacology resides in a relatively safe, clinically used cough suppressant like DM, we studied the anticonvulsant efficacy and acute behavioral toxicity of DM and a series of novel DM analogs in rodent models of convulsant behavior and neurological deficit. Although DM offered significant protection against spreading convulsions induced by MES and NMDA convulsions, its anticonvulsant efficacy was limited, a property likely related to its effect to lower seizure threshold at relatively high doses (see below). In contrast, DX, the major metabolite of DM, was a maximally efficacious, potent anticonvulsant against MES and NMDA convulsions which, unlike DM or the

DM analogs, was capable of increasing the seizure thresholds to flurothyl convulsions. Unfortunately, the anticonvulsant effects of DX were of relatively short duration and occurred at doses only slightly below those inducing neurobehavioral impairments. All three of the DM analogs studied were either more potent than DM and/or equipotent to DX against MES convulsions. Compared to DM, their lack of effect against flurothyl threshold convulsions probably accounts for their improved anticonvulsant efficacy. Compared to DX, their weaker potency against NMDA convulsions as well as their lack of rotarod impairing effects likely reflects their relative lack of affinity for the noncompetitive PCP site of the NMDA receptor complex. Significantly, the PI values for the DM analogs greatly exceeded those for DM, or reported for other standard anticonvulsants, in similar testing paradigms in rats (Loscher and Nolting, 1991). Therefore, the results of this study not only confirm that DM and the PCP-like drug DX have prominent anticonvulsant effects in rodent models of experimental epilepsy but, more importantly, demonstrate that dramatic improvement in potency, efficacy, duration of action and safety can be obtained by modification of the DM molecule.

DM was described originally as an anticonvulsant against MES convulsions in rats (Tortella and Musacchio, 1986) and subsequently was shown to possess anticonvulsant activity against a variety of preclinical experimental models of seizures (see Tortella *et al.*, 1989b). Whereas a recent case study described a considerable reduction in seizure frequency with DM in a patient suffering from medically refractory complex partial seizures (Wieser and Beck, 1992), the clinical efficacy of DM as a valuable antiepileptic drug in humans remains to be established (Fisher *et al.*, 1990). Although the PI of DM (table 1 and Leander *et al.*, 1988b) is well within the range of prototypical MES-selective anticonvulsant drugs including phenytoin, carbamazepine, phenobarbital and diazepam (Loscher and Nolting, 1991), its usefulness as a therapeutic drug in humans may be severely limited due to its metabolism to the PCP-like drug DX. Therefore, the importance of developing anticonvulsant analogs of DM with PI values at least similar to standard antiepileptic drugs, but whose mechanism of action is independent of metabolism to DX and distinct from that of DX or PCP, is indisputable.

Subjective observations of rat behavior revealed that none of the analogs produced overt sedation or ataxia when administered at the highest doses tested. More importantly, even minimal neurological deficit as defined by using the rotarod test (Loscher and Nolting, 1991) was nonexistent. Therefore, with PI values at least 4- to 5-fold greater than any prototypical anticonvulsant drug currently available one might surmise that these analogs represent a new class of safe and effective anticonvulsant drugs. A note of caution is warranted, however, as preclinical underestimation of neurobehavioral toxicity can result from a variety of factors (Loscher and Nolting, 1991), especially when determinations of PI values are not based on plasma or brain levels of the drugs in question, as was the case in the present study.

A critical finding of this study was our observation that the DM analogs were ineffective against flurothyl threshold convulsions. This result is important for two reasons. First, because the flurothyl convulsion model, like the metrazol test (Swinyard, 1969), classically screens for anticonvulsant drugs effective against the petit mal epilepsies (Adler, 1975), it would appear that the anticonvulsant spectrum of action of the DM

analog may be limited. This finding, however, is somewhat tempered by our results demonstrating that the DM analogs also protect against NMDA convulsions whereas other clinically effective compounds which block MES convulsions, such as phenytoin or phenobarbital, are ineffective against NMDA (Czuczwar *et al.*, 1985; Leander *et al.*, 1988a). Secondly, the proconvulsant effects of DM in the flurothyl seizure test, and the recognition of proconvulsant effects generally associated with high doses of anticonvulsant drugs such as phenytoin or carbamazepine (Loscher *et al.*, 1991), likely contribute to the limited efficacy found with DM in some seizure models. Therefore, as shown in the present study, it is possible that the improved efficacy obtained with the DM analogs is related to their failure to negatively influence seizure thresholds. Within this framework it will be important to evaluate the effects of the DM analogs against kindled seizures and other *in vivo* (genetically predisposed) and *in vitro* (cortical or hippocampal slice preparations) models of spontaneous epileptiform activity.

The efficacy of DM as an antagonist of NMDA convulsions in rodents (Ferkany *et al.*, 1988; Aram *et al.*, 1989; Aplan and Braitman, 1990), albeit weak, has been confirmed in the present study. The i.c.v. administration of DM, while effectively delaying the onset to NMDA convulsions, failed to antagonize the incidence of NMDA-induced convulsions. This limited effect of DM to antagonize NMDA convulsions may be explained, in part, by the results of binding studies which suggest that DM interacts with relatively low affinity with glutamate/NMDA binding sites (Craviso and Musacchio, 1983; Tortella *et al.*, 1989b), including the glycine modulatory site on the NMDA receptor complex (Newman *et al.*, 1992).

In contrast, the results of other binding analysis and competition studies have indicated that DM interacts with multiple high and low affinity DM sites as well as high affinity *sigma* sites (Klein and Musacchio, 1989; Zhou and Musacchio, 1991). Interestingly, similar to DM other highly selective *sigma*-1 ligands such as (+)-3-(hydroxyphenyl)-N-n-propylpiperone and (+)-pentazocine (Walker *et al.*, 1990) have been found to lower flurothyl seizure thresholds in rats (Echevarria *et al.*, 1990) and induce epileptiform EEG activity at high doses (F. C. Tortella, unpublished observations), suggesting that activation of a population of *sigma* receptors may propagate convulsant responses, rather than mediate anticonvulsant activity. Therefore, in view of the high affinity of DM for *sigma*-1 sites (*vide infra*), it is possible that the proconvulsant effect of DM measured in the flurothyl experiments and its limited efficacy against MES and NMDA convulsions, results from an interaction at this high affinity *sigma*-1 binding site. Importantly, other high affinity "anticonvulsant" DM ligands such as carbapentane and caramiphen (Tortella and Musacchio, 1986; Tortella *et al.*, 1988b; Leander, 1989; Aram *et al.*, 1989; Aplan and Braitman, 1990) also exhibit high relative affinities for *sigma*-1 sites (Walker *et al.*, 1990) and, likewise, lower flurothyl seizure thresholds (F. C. Tortella, unpublished observations).

Our assessment of the binding characteristics of DM and the DM analogs indicates that a functional relationship may exist between anticonvulsant activity and high affinity DM-site binding in the brain (Newman *et al.*, 1992). Importantly, the DM analogs have been shown to have negligible affinities for the [³H]thienylcyclohexylpiperidine or [³H]glycine sites on the NMDA receptor complex (Newman *et al.*, 1992). Therefore, it is unlikely that their ability to antagonize NMDA convulsions observed in the present study resulted from interactions with

noncompetitive NMDA ion channel receptors or the strychnine-insensitive glycine modulatory site, but rather may be due to some other modulatory action on the NMDA receptor-complex. Although a definitive determination of the receptor mechanism of action mediating the seizure protective properties of these compounds awaits a more detailed analysis, the results of these binding experiments suggest that a selective interaction with a high affinity DM site may be responsible. Additional studies are underway addressing this question, as well as the possible role of intracellular neuronal calcium dynamics (Carpenter *et al.*, 1988; Church *et al.*, 1991; DeCoster *et al.*, 1992), in the anticonvulsant mechanism of action for DM and the respective DM analogs.

Several results suggest that metabolism to DX is not a prerequisite for anticonvulsant activity. First, DM is anticonvulsant in *in vitro* slice preparations where metabolism to DX is unlikely (Aram *et al.*, 1989; Aplan and Braitman, 1990). Second, we have demonstrated in this study that minor modifications at position-3 of the 17-methylmorphinan molecule, producing compounds which might not be expected to be metabolized to DX, or do so only at a reduced rate (Newman *et al.*, 1992), can significantly improve the anticonvulsant pharmacology of DM. Substitution of the 3-methoxy group with the primary amine (AHN649) only slightly increased the potency of DM, but significantly improved the anticonvulsant efficacy, duration of action and PI. In turn, addition of steric bulk to the alkyl side chain as seen with the alkyl ether series of compounds (AHN1-036 and AHN1-037) significantly improved anticonvulsant potency without effecting neurological function. While it is possible that these alkyl ethers could be metabolized *via* dealkylation to DX, it is important to recognize that 1) none of the analogs were behaviorally similar to DX in the rat; 2) unlike DX they failed to raise seizure thresholds to flurothyl; 3) compared to DX, the respective analogs exhibited an improved duration of action with ANH649 continuing to manifest seizure protection as long as 4-hr postinjection; and 4) all the DM analogs were anticonvulsant after *i.c.v.* administration where metabolism in brain to DX is unlikely.

In conclusion, novel analogs of DM have been synthesized which exhibit improved potency, efficacy, duration of action and safety as potential anticonvulsant treatments. Because of their efficacy in the MES and NMDA models of experimental seizures, the DM analogs may represent a novel class of antiepileptic drugs for the treatment of generalized seizures of the grand mal type as well as partial (focal) seizures. Their excellent PI values clearly distinguish them from prototypical anticonvulsant agents currently in clinical use, as well as from PCP-like anticonvulsant agents including DX, ketamine and MK-801. Although the critical issue of tolerance development remains to be addressed, the results of the present study would seem to establish these DM analogs as potential drug development candidates for the treatment of epilepsy and possibly other neurodegenerative disorders such as stroke or brain/spinal cord trauma.

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