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Dextromethorphan Attenuates Ethanol Withdrawal Syndrome in Rats

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ERDEN, B. F., S. OZDEMIRCI, G. YILDIRAN, T. UTKAN, N. GACAR AND G. ULAK. *Dextromethorphan attenuates ethanol withdrawal syndrome in rats.* PHARMACOL BIOCHEM BEHAV **62**(3) 537–541, 1999.—The effects of dextromethorphan (DM), a noncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptors, have been investigated on ethanol withdrawal signs in rats. Ethanol (7.2% v/v) was given to rats in a liquid diet for 16 days. DM (10, 20, and 40 mg/kg) and saline were injected intraperitoneally at the third hour of ethanol withdrawal. DM (40 mg/kg) and ethanol dependent saline were also administered to ethanol naive rats. DM (40 mg/kg) did not produce any significant change in locomotor activity in ethanol naive rats. The effects of DM on locomotor activity and total ethanol withdrawal score were evaluated at the fourth and sixth hours of ethanol withdrawal. DM inhibited locomotor hyperactivity at these periods. DM also reduced total ethanol withdrawal score from the fourth hour to the sixth hour, and it significantly decreased audiogenic seizures. Seizure susceptibility after chronic ethanol exposure may be dependent upon sensitization or upregulation of NMDA processes and NMDA receptors. Our results suggest that inhibition of NMDA receptors by DM alleviates signs of ethanol withdrawal.

Dextromethorphan *N*-Methyl-D-aspartate Ethanol withdrawal Audiogenic seizure

THE discontinuation of chronic administration of central depressant drugs such as ethanol is associated with excitatory withdrawal signs (5). Although ethanol withdrawal syndrome in humans (24) and rats (13,28) has been well described, mechanisms underlying physical dependence to ethanol or the ethanol withdrawal syndrome are poorly understood. However, recent studies indicate the importance of increased excitatory amino acid neurotransmission in the development of the ethanol withdrawal syndrome (6,21).

The common antitussive drug dextromethorphan (DM) and its active metabolite dextrorphan can act as noncompetitive *N*-methyl-D-aspartate (NMDA) antagonists (3). DM, as well as dextrorphan, is virtually devoid of opioid activity (10). They have no antinociceptive action through any of the opioid receptors and no addiction liability (10). The ability of DM seems to antagonize NMDA receptor activation (19). DM has previously been reported to antagonize the precipitated abstinence syndrome signs in physically morphine-dependent rats (10). In addition, the successful use of DM in the treatment of opiate-addicted outpatients is considered as supporting evidence that effects of DM are at the levels of NMDA receptors (8,9,23). It has been recently shown that DM protects against hypoxia–ischemia cerebral infarction in a rat model (19) and humans (2). DM has an established safety record in humans at antitussive doses, and thus appears to be an attractive compound for clinical investigation.

The present study was designed to confirm the hypothesis that the various doses of DM attenuate the behavioral symptoms of ethanol withdrawal signs including audiogenic seizures in rats.

METHOD

Animals and Laboratory

Adult male Wistar rats (210–290 g at the beginning of the experiments) were used. They were placed in a quiet and temperature- and humidity–controlled room (21 ± 4°C and 60 ± 9%, respectively) in which 12–12 h light–dark cycle was maintained (08:00–20:00 h light).

Drugs

Dextromethorphan hydrobromide (Sigma Chemical, St. Louis, MO) was dissolved in saline. Dextromethorphan and

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saline were injected intraperitoneally at a volume of 0.5 ml/200 g body weight. Drug stocks were prepared fresh on the morning of each experiment. The dose chosen was adapted from other studies that have examined the effect of dextromethorphan on the pharmacokinetics and behavioral response (31), on discriminative stimulus effects (4), and on cerebral infarction in hypoxia–ischemia (19) in vivo.

Experimental Procedures

Rats were individually housed in metal cages. Ethanol (7.2% v/v) was given to rats in a modified liquid diet for 16 days as previously described (28). Liquid diet was prepared daily and given to the rats at the same time of day (10:00 h). The weights of the rats were recorded every day, and the daily ethanol intake was measured and expressed as grams per kilogram per day. Ethanol-naive rats were pair fed on an isocaloric liquid diet containing sucrose as a caloric substitute for ethanol.

At the end of the exposure to 7.2% ethanol-containing liquid diet, ethanol was withdrawn from the diet by replacing the diet with one that did not contain ethanol at 10:00 h. The ethanol-dependent rats were assigned into four groups. DM (10, 20, and 40 mg/kg), and saline were injected intraperitoneally at the third hour of ethanol withdrawal testing.

The rats were then observed for 4 min at the fourth and sixth hour of the ethanol-withdrawal period. These time periods were short, and only part of the full behavioral withdrawal syndrome was assessed during these time points. At each observation time, rats were assessed simultaneously for the following behavioral conditions: locomotor activity, body posture, gait, agitation, tail stiffness, tremor, stereotyped behavior, and wet dog shakes. We measured locomotor activity automatically with a computerized on-line open field test ($40 \times 40 \times 35$ cm box; May, Commat, Ankara, Turkey). Rats were put in the open field arena 30 s before starting the experiment. A printout for each session showed the ambulatory movements of the animals in the open-field box. The distance travelled in centimeters by the rats in the horizontal locomotor activity was analyzed. Wet dog shakes and tremors were assessed by incidence. Wet dog shake behavior was considered positive if it occurred at least three times during the observation period. Tremor was determined after lifting rats vertically by the tail: positive was assigned to rats showing clearly distinct forelimb tremor when they were rotated 180° around axis of tail. In the study, grooming, sniffing, head weaving, gnawing, and chewing were observed as major stereotypes behaviors during the ethanol withdrawal. Stereotypic behaviors, abnormal posture and gait, agitation, and tail stiffness were scored using a rating scale (Table 1).

At the sixth hour of the withdrawal testing, rats were exposed to an audiogenic stimulus (100 dB) for 1 min (17). The intensity of the seizures was scored as follows: seizures were rated on a five-point scale ranging from 1 to 5. A score of 1 was assigned to rats showing only wild running. The rats showing tonic and tonic–clonic seizures in addition to wild running were given scores of 2 and 3, respectively. A score of 4 was assigned to the rats with longer lasting periodic (>90 s) tonic–clonic seizures. A score of 5 was given if mortality occurred.

The intensity of the parameters was expressed as a median value. To calculate the total ethanol withdrawal score, the behavioral parameters were expressed as percent incidence and converted into scores ranging from 1 to 5 (10–20%: 1; 30–40%: 2; 50–60%: 3; 70–80%: 4; 90–100%: 5) (28). Then the median values of each behavior were summed for an individual observation period.

TABLE 1

RATING SCALE FOR SOME BEHAVIOR SIGNS INDUCED BY ETHANOL WITHDRAWAL IN RATS

Signs	Scoring
Stereotyped behaviors*	1: rats showing only one stereotyped behavior
	2: two stereotyped behavior
	3: three stereotyped behavior
	4: four stereotyped behavior
	5: all of stereotyped behavior
Agitation	1: rats showing mild or moderate irritability
	2: very irritable
	3: handling vocalization and moderately aggressive
	4: handling vocalization and very aggressive
	5: spontaneous vocalization and very aggressive
Tail stiffness	1: mild tail rigidity
	2: moderate tail rigidity
	3: tail rigidity but mildly flexible during ambulation
	4: tail rigid and not flexible during ambulation
	5: tail very rigid and not flexible during ambulation
Abnormal posture	1: mild head-down, back-hunched
	2: moderate head-down, back-hunched
	3: prominent head-down, back-hunched
	4: in addition hind legs wide apart
	5: in addition fore limbs apart
Abnormal gait	1–2: mild difficulty ambulating and rearing normal
	3–4: moderate difficulty ambulating and rearing
	5: prominent difficulty ambulating and no rearing

*Grooming, sniffing, head weaving, gnawing, and chewing.

All experiments were carried out at the same time every day during the light period. All the ratings were done by experimentally naive observers. The experiments reported in this study have been carried out in accordance with the Declaration of Helsinki. Ethical approval was granted by the Kocaeli University Ethics Committee (Kocaeli, Turkey).

Blood Ethanol Determination

Blood ethanol levels were determined in the two groups of ethanol-receiving rats run in parallel to the ethanol dependent behavioral test group ($n = 5$ for each group). Concentrations were determined by headspace gas chromatography method (11). We took blood samples by intracardiac puncture from the rats under very light ether anesthesia. Samples were taken before removing ethanol from the liquid diet in one group and at the sixth hour following the ethanol withdrawal in the other.

Ethanol Naive Control Experiments

DM (40 mg/kg) and saline were administered to two groups of naive Wistar rats fed an isocaloric liquid diet with-

out ethanol ($n = 10$ for each group). Locomotor activity was recorded as experimental groups. The animals were observed for the same signs of ethanol withdrawal as in ethanol-dependent groups.

Statistical Analysis

Changes in locomotor activity were compared by unpaired Student's *t*-test. Total ethanol withdrawal scores in the different groups were compared by using Mann–Whitney *U*-tests. Comparisons of the incidence and intensity of the audiogenic seizures in different groups were done by Fischer's exact test and Mann–Whitney *U*-test, respectively. The level of significance was set at $p < 0.05$.

RESULTS

Ethanol Consumption and Blood Ethanol Levels

Daily ethanol consumption of the rats was in a range of 11.8–14.5 g/kg. Blood ethanol levels were 201.0 ± 15.6 and 10.4 ± 2.5 mg/dl (mean ± SEM) at the beginning of the withdrawal period and at the sixth hour of ethanol withdrawal, respectively.

Behavioral Changes During Ethanol Withdrawal

A locomotor hyperactivity was observed in the ethanol-dependent group during the withdrawal testing period (Table 2). Other behavioral signs of ethanol withdrawal syndrome such as abnormal posture and gait, agitation, wet dog shakes, tail stiffness, tremor, and stereotyped behaviors appeared during the whole observation period. The total ethanol withdrawal score was the highest at the sixth hour of ethanol withdrawal in the ethanol-dependent group (Fig. 1, white bars).

Effects of DM on Locomotor Hyperactivity Induced by Ethanol Withdrawal

DM reduced the locomotor hyperactivity seen in the ethanol-dependent group. The reduction became more significant in the 40 mg/kg DM group compared with the ethanol-dependent control group (Table 2).

Effects of DM on Total Ethanol Withdrawal Score

DM dose dependently reduced the total ethanol withdrawal score compared with the ethanol-dependent group (Fig. 1). The total ethanol withdrawal score was significantly lower than in the ethanol-dependent control rats. The results are for DM 10 mg/kg ($U = 19.0$, p = 0.018), for DM 20 mg/kg ($U = 3.5$, $p = 0.0003$), and for DM 40 mg/kg ($U = 2.5$, $p = 0.0002$) at the fourth hour of ethanol withdrawal; for DM 10 mg/kg ($U = 2.0$, $p = 0.0003$), for DM 20 mg/kg ($U = 0.0$, $p = 0.0001$), and for DM 40 mg/kg ($U = 0.0$, $p = 0.0001$) at the sixth hour of ethanol withdrawal.

Effects of DM on Audiogenic Seizures

Exposure to an audiogenic stimulus at the sixth hour of the ethanol withdrawal precipitated seizures with an incidence of 80% in the ethanol-dependent group. DM dose dependently decreased the incidence and intensity of the audiogenic seizures (Table 3).

Observations in Ethanol Naive (Nondependent) Rats

Locomotor activities of the ethanol naive rats were significantly lower compared with the ethanol-dependent group (Table 2). These rats did not exhibit any ethanol withdrawal signs during the observation period. Under our experimental conditions, DM (40 mg/kg) did not produce any significant change in locomotor activity in the ethanol-naive rats.

TABLE 2

INFLUENCE OF DM ON THE LOCOMOTOR ACTIVITY AND EXPLORATION IN RATS

Drug	Locomotion (counts ± SEM/4 min)	Locomotion (cm ± SEM/4 min)
Ethanol naive ($n = 10$)		
4th h	13.1 ± 1.5	68.0 ± 4.3
6th h	9.8 ± 1.1	60.4 ± 3.5
DM 40 mg/kg naive ($n = 10$)		
4th h	15.4 ± 1.3	72.9 ± 5.4
6th h	11.6 ± 1.0	68.5 ± 6.7
Ethanol dependent ($n = 10$)		
4th h	20.4 ± 2.0	106.6 ± 11.6
6th h	22.5 ± 3.2	173.1 ± 19.3
DM 10 mg/kg ($n = 10$)		
4th h	23.9 ± 3.5	137.3 ± 34.4
6th h	11.6 ± 2.7*	68.5 ± 18.8*
DM 20 mg/kg ($n = 11$)		
4th h	16.8 ± 2.8	70.7 ± 14.5*
6th h	17.2 ± 2.9	98.1 ± 12.9*
DM 40 mg/kg ($n = 11$)		
4th h	11.3 ± 4.6*	69.3 ± 21.8*
6th h	10.3 ± 2.6*	101.7 ± 14.9*

The influence of DM on the horizontal distance traveled by the rat and the locomotor counts in the 4-min monitoring period.

*$p < 0.05$ significantly different from ethanol-dependent rats.

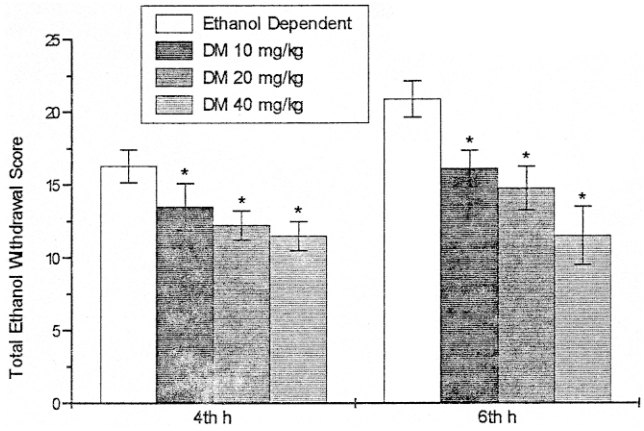

FIG. 1. Effects of DM on total ethanol withdrawal score at the fourth and sixth hours of ethanol withdrawal. DM was injected at the third hour of ethanol withdrawal. Values are the medians ± semi-interquartile ranges of individual withdrawal scores for 10–11 animals/group (h = hour; *$p < 0.05$, Mann–Whitney *U*-tests, significantly different from ethanol dependent rats).

TABLE 3

EFFECTS OF DM ON THE INCIDENCE AND INTENSITY OF THE AUDIOGENIC SEIZURES IN ETHANOL-DEPENDENT RATS

Treatment	Incidence (%)	Intensity (Median Score)
Ethanol dependent ($n = 10$)	80	3.3
DM 10 mg/kg ($n = 10$)	50*	2.2*
DM 20 mg/kg ($n = 11$)	36*	1.5*
DM 40 mg/kg ($n = 11$)	9*	N/A

N/A (Not available): There were insufficient rats with audiogenic seizures in this group. Rats were pretreated with DM 3 h prior to the time of assessment of the seizures (in the 2-min monitoring period).

*$p < 0.05$ significantly different from ethanol-dependent rats.

DISCUSSION

Daily ethanol consumption, ranging from 11.8 to 14.5 g/kg throughout 16 days, was sufficient to produce physical dependence in rats. As we also observed in the present study, high average blood ethanol levels (201 mg/dl) just before the ethanol withdrawal demonstrate adequate ethanol consumption (13,26–28).

In light of the experimental results mentioned earlier, we tested the ability of DM to prevent and/or block of ethanol withdrawal signs in rats. In ethanol-naive rats, DM by itself had no effect. But in ethanol-dependent rats, DM produced a prominent inhibitory effect on signs of ethanol withdrawal.

DM exhibited inhibitory effects on the locomotor hyperactivity and total ethanol withdrawal score for ethanol-dependent rats in our study. Also, we have found an inhibitory action of DM on the audiogenic seizures. This sign is considered as the most reliable and easily quantifiable element of ethanol withdrawal score in rats (1,13). These findings are in agreement with other studies that MK-801, an other noncompetitive NMDA receptor antagonist, inhibits alcohol withdrawal seizures in rats (17). CGP39551, a competitive NMDA receptor antagonist, given immediately after ethanol withdrawal, was protective against hyperexcitability produced by withdrawal from chronic ethanol treatment in mice (20). After chronic ethanol exposure, withdrawal signs may be particularly dependent upon sensitization or upregulation of NMDA processes (17). Lovinger et al. (12) also find that ethanol inhibits NMDA operated currents in hippocampus and dorsal root ganglion.

Other studies have shown that DM inhibited the severity of the morphine withdrawal syndrome in dependent rats by decreasing the intensity of certain signs such as jumping and teeth chattering (7,10,15). These studies suggested that DM attenuates and/or prevents the manifestation of abstinence by the blockade of the NMDA receptors. Several studies have appeared in the literature in support of opiate–ethanol interaction; especially, ethanol and opioids may share some common elements in their effects (14).

Generally, we know that DM does not possess the central nervous system effects of other opiates in humans (i.e., analgesia, respiratory depression, and abuse liability or psychotomimetic properties). DM is still used as a nonprescription cough suppressant, but its anticonvulsant and neuroprotective properties have been demonstrated (25). Several antipsychotic and antidepressant drugs and certain anticonvulsants also have been shown to interact with the high affinity DM site (25).

Consequently, we saw that DM attenuates ethanol withdrawal signs in rats. Other NMDA receptor antagonists may be also worthy of mention in the treatment of ethanol-addicted people. Unfortunately, most of these agents are not suitable for oral and parenteral administration because they do not easily cross the blood–brain barrier and have unacceptable side effects (19). On the other hand, DM, administered up to the dose of 100 mg/kg IP, failed to induce phencyclidine-like behavioral effects in rats (22). Some limited and sporadic abuse of DM has been reported (29,30) as well as a case of toxic psychosis and exacerbation of preexisting schizophrenia (18). There have even been sporadic reports of DM abuse and bulimia, but not enough to warrant its international control as a narcotic (16). Additional studies to evaluate the use of DM in the treatment of ethanol withdrawal will be required.

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