Pharmacology Biochemistry and Behavior, Vol. 45, pp. 755–757, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

BRIEF COMMUNICATION

Ethanol- and Diazepam-Withdrawal Hyperlocomotion is Not Due to 5-HT_3 Receptor Stimulation

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Received 9 November 1992

MEHTA, A. K. AND M. K. TICKU. *Ethanol- and diazepam-withdrawal hyperlocomotion is not due to 5-HT_3 receptor stimulation.* PHARMACOL BIOCHEM BEHAV **45**(3) 755–757, 1993.—The effect of 5-HT_3 receptor antagonists such as ondansetron, ICS 205-930, MDL 72222, metoclopramide, and zacopride was investigated on the ethanol as well as diazepam withdrawal phenomena in the present study. There was a significant increase in locomotor activity in the ethanol- as well as diazepam-withdrawn rats. The treatment of rats with 5-HT_3 receptor antagonists during withdrawal phase did not modify the effect. The ethanol-withdrawn rats were more sensitive to pentylenetetrazole (PTZ)-induced convulsions as compared to control animals. 5-HT_3 receptor antagonists did not attenuate the increased sensitivity of ethanol-withdrawn rats to PTZ. These observations indicated that 5-HT_3 receptor antagonists are ineffective in attenuating hyperlocomotor activity following abrupt termination of chronic administration of ethanol or diazepam, and increased sensitivity to PTZ in the ethanol-withdrawn rats.

5-HT_3 Receptor antagonists Ethanol Diazepam Hyperlocomotor activity Convulsions

BENZODIAZEPINES (BZ) are clinically effective as anxiolytics and antiepileptics with a broad spectrum of pharmacological actions. Increased anxiety is one of the abstinence signs of withdrawal from BZ (2,4). The exact mechanism of BZ withdrawal abstinence signs is not yet known. Similarly, the exact mechanisms underlying the dependence and withdrawal syndromes of ethanol, a substance whose pharmacological effects resemble those of drugs such as BZ and barbiturates that facilitate the GABAergic transmission, are not fully understood. It has been reported recently that ondansetron, a 5-HT_3 receptor antagonist, attenuates the BZ-withdrawal effects on body weight (6), food intake (6), and aversive behavior (1) in rats. However, ICS 205-930, another 5-HT_3 receptor antagonist, did not alleviate BZ-withdrawal effects on body weight and food intake (5). Recently, a few reports have appeared in the literature which indicate the interaction between ethanol and 5-HT_3 receptors (3,7,10). It has been reported that ethanol potentiates 5-HT_3 receptor-mediated ion current in whole-cell patch-clamp recordings from NCB-20 neuroblastoma cells (7). On the other hand, 5-HT_3 receptor antagonists have been reported to attenuate the effects of ethanol on extracellular dopamine (10) and the discriminative stimulus properties of ethanol (3). It has also been reported that ondansetron antagonizes the aversive behavior caused by withdrawal from ethanol (1). In view of these reports, we investigated the effects of various 5-HT_3 receptor antagonists such as ondansetron, ICS 205-930, MDL 72222, metoclopramide, and zacopride on ethanol- and diazepam-withdrawal syndromes in rats.

METHOD

Animals

Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 200–220 g were used. The animals were maintained at a constant room temperature (25 °C) on a 12L : 12D cycle. They were allowed free access to both food and water.

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TABLE 1

EFFECT OF 5-HT_3 RECEPTOR ANTAGONISTS ON HYPERLOCOMOTOR ACTIVITY FOLLOWING WITHDRAWAL FROM CHRONIC TREATMENT OF RATS WITH ETHANOL

Treatment mg/kg, IP; Three Times a Day for 1 Day	*n*	Locomotor Activity During a 10-Min Period (Mean ± SD)
Control	6	437 ± 41
Ethanol withdrawal	8	597 ± 30*
+ Ondansetron HCl.$2H_20$ (0.01)	6	594 ± 36^{NS}
+ Ondansetron HCl.$2H_20$ (0.1)	6	591 ± 36^{NS}
+ ICS 205-930 (0.1)	6	603 ± 36^{NS}
+ ICS 205-930 (1)	6	572 ± 46^{NS}
+ MDL 72222 (0.1)	5	595 ± 29^{NS}
+ MDL 72222 (1)	6	607 ± 23^{NS}
+ Metoclopramide HCl (0.1)	5	577 ± 48^{NS}
+ Metoclopramide HCl (1)	6	627 ± 48^{NS}
+ Zacopride HCl (0.1)	6	586 ± 34^{NS}
+ Zacopride HCl (1)	7	617 ± 19^{NS}

The locomotor activity was recorded following 15 min of the administration of the last dose of 5-HT_3 receptor antagonist or vehicle.
*$p < 0.05$ as compared to control group.
^{NS} = not significant as compared to ethanol-withdrawn group.

Locomotor Activity and Convulsions in the Ethanol-Withdrawn Rats

The rats were intoxicated by an intragastric intubation method for 6 days (8). Briefly, at the beginning of the experiment, a priming dose of ethanol of 5 g/kg (20% w/v in normal saline) was administered to all animals. Following this, doses (9–15 g/kg in three doses over 24-h period) were adjusted individually for each animal according to the presence or absence of ataxia and loss of righting reflex. The preliminary experiments revealed that the withdrawal effects peaked following 24 h of the last dose of ethanol administration in rats. Hence, the ethanol-withdrawn rats were tested 24 h after the last dose of ethanol for subsequent experiments. The effect of 5-HT_3 receptor antagonists was investigated by replacing ethanol by 5-HT_3 antagonists during the ethanol-withdrawal phase (three doses of 5-HT_3 receptor antagonists administered intraperitoneally over the 24-h period). The doses of various 5-HT_3 receptor antagonists were selected based on previous studies (1,3,5,6). The control rats received vehicle.

The locomotor activity and pentylenetetrazole (PTZ)-induced convulsions were studied in different groups of rats following 15 min of the administration of the last dose of 5-HT_3 receptor antagonist or vehicle. For locomotor activity, rats were individually placed in the cages, and the locomotor activity was recorded using an activity meter (Stoelting, USA) for a 10-min period. For the convulsion study, each animal was observed individually for the time of onset of convulsion as well as for mortality.

Locomotor Activity in the Diazepam-Withdrawn Rats

Another set of rats were injected with diazepam (suspended in double distilled water containing a drop of Tween 80 per 10 ml), 10 mg/kg, IP twice a day for 2 weeks followed by 12 mg/kg, IP twice a day during the third week. The preliminary experiments revealed that the withdrawal effects peaked following 3 days of the last dose of diazepam administration in rats. Hence, the diazepam-withdrawn rats were tested 3 days after the last dose of diazepam for subsequent experiments. The effect of 5-HT_3 receptor antagonists was investigated by replacing diazepam by 5-HT_3 antagonists during the BZ-withdrawal phase (two doses of 5-HT_3 antagonists administered intraperitoneally over a 24-h period for 3 days). The doses of 5-HT_3 receptor antagonists were selected based on earlier reports (1,3,5,6). The control rats received vehicle. The locomotor activity was recorded, following 15 min of the administration of the last dose of 5-HT_3 receptor antagonist or vehicle, as a paradigm to investigate the efficacy of 5-HT_3 receptor antagonists in BZ-withdrawn rats as described above.

Statistics

The data are expressed as mean ± SD. Each group consisted of a minimum of five animals. The significance of differences between different groups of data was analyzed using Student's *t*-test.

RESULTS

There was a significant increase in locomotor activity in ethanol-withdrawn rats. The treatment of rats with 5-HT_3 receptor antagonists during the ethanol-withdrawal phase did not attenuate hyperlocomotor activity due to ethanol-withdrawal (Table 1) despite a slight but insignificant depressant effect per se of these drugs on locomotor activity in control rats (data not shown). PTZ (30 mg/kg, IP) induced mild clonic–tonic seizures of short duration, with no mortality, in two out of six control rats. Conversely, the ethanol-withdrawn rats were very sensitive to the action of PTZ because it produced severe tonic seizures (8/8) and 50% (4/8) mortality in a dose of 30 mg/kg, IP (Table 2). 5-HT_3 receptor antagonists did not attenuate the increased sensitivity of ethanol-withdrawn rats to PTZ (Table 2). Furthermore, 5-HT_3 receptor antagonists did not elicit any significant effect per se on PTZ-induced convulsion in control rats.

TABLE 2

EFFECT OF 5-HT_3 RECEPTOR ANTAGONISTS ON PTZ-INDUCED CONVULSIONS IN ETHANOL-WITHDRAWN RATS

	Effect of PTZ (30 mg/kg, IP)	
Treatment mg/kg, IP; Three Times a Day for 1 Day	Onset of Convulsions (Mean s ± SD)	Mortality (*n*)
Control	90 ± 8 (2/6)	0/6
Ethanol withdrawal	73 ± 13 (8/8)	4/8*
+ Ondansetron HCl.$2H_20$ (0.01)	76 ± 10 (6/6)	3/6^{NS}
+ Ondansetron HCl.$2H_20$ (0.1)	75 ± 10 (5/5)	2/5^{NS}
+ ICS 205-930 (0.1)	66 ± 10 (6/6)	3/6^{NS}
+ ICS 205-930 (1)	64 ± 14 (6/6)	4/6^{NS}
+ MDL 72222 (0.1)	61 ± 10 (5/5)	3/5^{NS}
+ MDL 72222 (1)	70 ± 16 (6/6)	4/6^{NS}
+ Metoclopramide HCl (0.1)	71 ± 12 (5/5)	2/5^{NS}
+ Metoclopramide HCl (1)	63 ± 15 (6/6)	4/6^{NS}
+ Zacopride HCl (0.1)	65 ± 6 (6/6)	3/6^{NS}
+ Zacopride HCl (1)	64 ± 14 (7/7)	5/7^{NS}

PTZ-induced convulsions were recorded following 15 min of the administration of the last dose of 5-HT_3 receptor antagonist or vehicle.
*$p < 0.05$ as compared to control group.
^{NS} = not significant as compared to ethanol-withdrawn group.

TABLE 3

EFFECT OF 5-HT$_3$ RECEPTOR ANTAGONISTS ON LOCOMOTOR ACTIVITY IN DIAZEPAM-WITHDRAWN RATS

Treatment mg/kg, IP; Twice a Day for 3 Days	n	Locomotor Activity During a 10 Min-Period (Mean ± SD)
Control	6	464 ± 36
Diazepam withdrawal	6	693 ± 54*
+ Ondansetron HCl.2H$_2$0 (0.01)	6	683 ± 41^{NS}
+ Ondansetron HCl.2H$_2$0 (0.1)	6	686 ± 44^{NS}
+ ICS 205-930 (0.1)	5	643 ± 30^{NS}
+ ICS 205-930 (1)	5	664 ± 46^{NS}
+ MDL 72222 (1)	5	682 ± 35^{NS}
+ MDL 72222 (5)	5	688 ± 31^{NS}
+ Metoclopramide HCl (1)	5	669 ± 47^{NS}
+ Metoclopramide HCl (5)	5	676 ± 43^{NS}
+ Zacopride HCl (0.1)	5	675 ± 23^{NS}
+ Zacopride HCl (1)	5	681 ± 38^{NS}

The locomotor activity was recorded following 15 min of the administration of the last dose of 5-HT$_3$ receptor antagonist or vehicle.
*$p < 0.05$ as compared to control group.
^{NS} = not significant as compared to diazepam-withdrawn group.

On abrupt termination of diazepam treatment, the rats exhibited a marked hyperlocomotor activity on the third day of withdrawal. The treatment of rats with 5-HT$_3$ receptor antagonists during the BZ-withdrawal phase did not modify the effect (Table 3) in spite of the fact that these drugs had a slight but insignificant depressant effect per se on locomotor activity in control rats.

DISCUSSION

There are reports indicating the role of 5-HT$_3$ receptors in the pharmacological effects of ethanol (1,3,7,10). It has been speculated that 5-HT$_3$ receptor antagonists may be useful as therapeutic intervention agent for ethanol-related problems. However, 5-HT$_3$ receptor antagonists such as ondansetron, ICS 205-930, MDL 72222, metoclopramide, and zacopride failed to reverse hyperlocomotor activity in ethanol-withdrawn rats in the present study. Furthermore, these agents did not modify the increased sensitivity of ethanol-withdrawn rats to PTZ-induced convulsions. These findings indicate that 5-HT$_3$ receptor antagonists may not be able to reverse all the effects associated with ethanol-withdrawal. In accordance with our findings, there is a recent preliminary report indicating that MDL 72222, a 5-HT$_3$ receptor antagonist, failed to reverse the ethanol-withdrawal effects on the open arm and total arm activity of rats as well as on the threshold dose for PTZ discrimination in rats (9).

In the present study, the rats exhibited hyperlocomotor activity on abrupt termination of chronic diazepam treatment, an index of increased anxiety. This observation is consistent with earlier reports (2,4). Although ondansetron, a 5-HT$_3$ receptor antagonist, is reported to attenuate the BZ-withdrawal effect on body weight (6), food intake (6), and aversive behavior (1) in rats, 5-HT$_3$ receptor antagonists such as ondansetron, ICS 205-930, MDL 72222, metoclopramide, and zacopride failed to alleviate diazepam-withdrawal hyperlocomotor activity in rats in the present study. However, this finding is consistent with another report that ICS 205-930 is ineffective in alleviating BZ-withdrawal effects (5).

In conclusion, 5-HT$_3$ receptor antagonists such as ondansetron, ICS 205-930, MDL 72222, metoclopramide, and zacopride were ineffective in reversing the hyperlocomotor activity and increased sensitivity to PTZ in case of ethanol, and the hyperlocomotor activity in case of diazepam following withdrawal from chronic drug administration. Thus these agents may not be effective in reversing the ethanol- and diazepam-withdrawal hyperlocomotion.

ACKNOWLEDGEMENTS

We thank Mrs. Sadie Phillips for excellent secretarial assistance. This work was supported by funds from NINCDS grant NS-15339 and NIAAA grant AA04090.

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