Pharmacology Biochemistry & Behavior, Vol. 34, pp. 769–778. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

The Effects of Ondansetron (GR38032F) in Rats and Mice Treated Subchronically With Diazepam

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Received 21 July 1989

COSTALL, B., B. J. JONES, M. E. KELLY, R. J. NAYLOR, N. R. OAKLEY, E. S. ONAIVI AND M. B. TYERS. *The effects of ondansetron (GR38032F) in rats and mice treated subchronically with diazepam.* PHARMACOL BIOCHEM BEHAV **34**(4) 769–778, 1989.—Using rat and mouse models of aversive behaviour, we have further investigated the properties of the 5-HT_3 receptor antagonist ondansetron (GR38032F) that are relevant to its proposed use as an anxiolytic agent. Tolerance to the disinhibitory properties of diazepam was readily demonstrated in the social interaction test in the rat, but did not occur after subchronic treatment with ondansetron. In both the light/dark exploration test in mice and the social interaction test in rats, withdrawal from subchronic treatment with diazepam increased behavioural suppression, whereas this was not observed with ondansetron. The behavioural suppression and weight loss induced by either the withdrawal of diazepam or the administration of the benzodiazepine receptor antagonist, flumazenil, in animals treated subchronically with diazepam, was prevented or antagonised by diazepam or ondansetron. Buspirone was ineffective. It is concluded that, in rats and mice, tolerance to the disinhibitory effects of ondansetron does not occur, that withdrawal from subchronic treatment with ondansetron is not associated with any behavioural disturbances and that ondansetron is highly effective in preventing the behavioural suppression and weight loss following withdrawal from subchronic diazepam treatment. These data suggest that ondansetron may have major therapeutic advantages over currently available anxiolytic agents, particularly in patients who have previously received prolonged benzodiazepine therapy.

5-HT_3 receptor antagonist Ondansetron Diazepam Aversive behaviour Mouse Rat

DRUG treatment with the benzodiazepines is the major therapy for anxiety where the effectiveness of the treatment and lack of toxicity has encouraged an extensive medical usage for some 25 years. However, more recently, a growing literature attests to the development of dependence on these drugs and a withdrawal syndrome following cessation of treatment, where the psychological and physiological manifestations of anxiety (e.g., irritability, tension, restlessness, foreboding, tremor, headache, sweating and insomnia) pose a particular difficulty in the majority of patients [see review by Marks (12)]. The rate of development and intensity of the syndrome appears to be related to the particular benzodiazepine used, its half-life, the dose and duration of treatment.

The problems that have resulted from the use and abuse of the benzodiazepines emphasise the importance of identifying anxiolytic agents which lack abuse and physical dependence liability. The present study was designed to investigate, in mouse and rat models of suppressed behaviour, whether cessation of subchronic treatment with the 5-HT_3 receptor antagonist, ondansetron (2), a potential anxioytic drug (11), causes the development of tolerance or any overt withdrawal behaviours. Furthermore, studies have been carried out to determine the effects of ondansetron on the behavioural changes and weight loss induced by cessation of subchronic treatment with diazepam.

METHOD

Experiments in Mice

The light/dark aversion test in the mouse has been described in detail previously (6). Male albino BKW mice (Bradford strain), 25–30 g, were used throughout the studies. Mice were housed in groups of 10 in conditions of constant temperature (21°C) and controlled lighting (dark period 07.00–19.00 hr) and fed ad lib on a standard laboratory chow. Water was available in the living cages at all times.

Tests were conducted between 13.00 and 18.00 hr in a quiet darkened room illuminated with a red light. Mice were taken from a dark holding room in a dark container to the dark testing room where, after a 1-hour period of adaptation to the new environment, they were placed into the test box. The metal test box (45 × 27 × 27 cm high) was positioned on a bench 1 m above floor level. The box was open-topped and the base was lined into 9 cm squares, two-fifths painted black and illuminated by red light

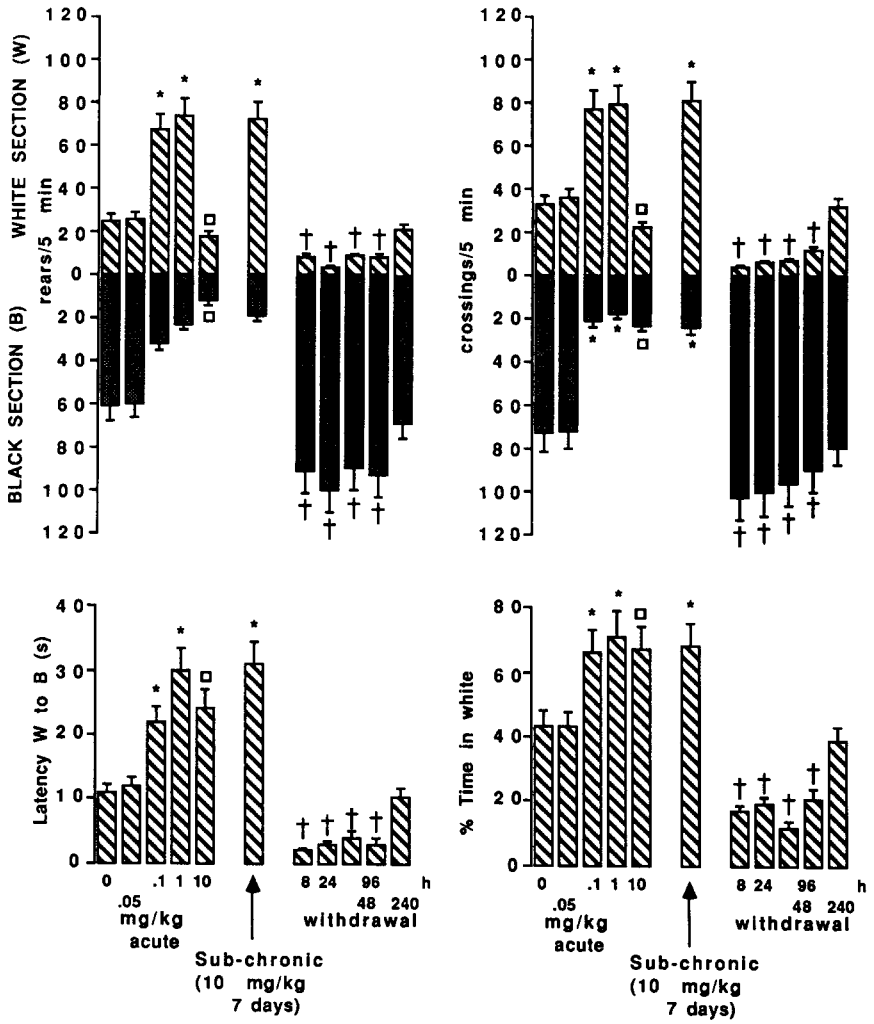

FIG. 1. The effects of diazepam given acutely, subchronically, or withdrawn from subchronic treatment, in the light/dark exploration test in mouse. Testing was carried out 45 min after acute dosing, 45 min after the first dose on the 7th day of treatment (subchronic) and then at five different times between 8 and 240 hr of withdrawal from subchronic treatment. n = 8 per group. *$p<0.001$ for increased exploration in the light and delayed latency for initial movement from the light, †$p<0.001$ for decreased exploration in the light and reduced latency (comparison to vehicle controls). Naive mice were used to obtain each histogram and vehicle control responses were determined on all test days. On no occasion did vehicle control values differ significantly from that presented. □ Indicates sedation. Standard errors of the means are given: for % time spent in the light these were calculated from original data.

(1 × 60 W, 0 lux) and partitioned from the remainder of the box which was painted white and brightly illuminated with a 1 × 60 W (400 lux) light source, the red and white lights being located 17 cm above the box. The compartments were connected by an opening 7.5 × 7.5 cm located at floor level in the centre of the partition. Mice were placed into the centre of the white, brightly lit area and the operator withdrew from the room. The mice were observed by remote video recording and four behaviours were noted, (a) the number of exploratory rearings in the light and dark sections, (b) the number of line crossings in the light and dark sections, (c) the time spent in the light and dark areas and (d) the latency of the initial movement from the light to the dark area.

Experimental Design

Diazepam and ondansetron were given acutely (45 min pre-treatment) to establish the dose ranges for inducing preference for the light section of the test box. Supramaximal doses (10 mg/kg IP b.i.d. diazepam, 0.1 mg/kg IP b.i.d. ondansetron) were then given repeatedly for 7 days (dosing at 08.00 and 20.00 hr) and subsequently withdrawn: behavioural consequences of withdrawal were determined at 8, 24, 48, 96 and 240 hr. The influence of ondansetron on the increased aversion for the light compartment caused by withdrawing from the subchronic treatment with diazepam was investigated, firstly, by determining the dose range over which behavioural effects of diazepam withdrawal could be inhibited (24-hr withdrawal only) and, secondly, selecting an effective dose of ondansetron (10 μg/kg IP b.i.d.) for administration throughout the 240-hr diazepam withdrawal period to determine whether the diazepam withdrawal phenomenon could be inhibited at all measurement times. Similar experiments were carried out using buspirone (1 mg/kg IP b.i.d.). The effect on

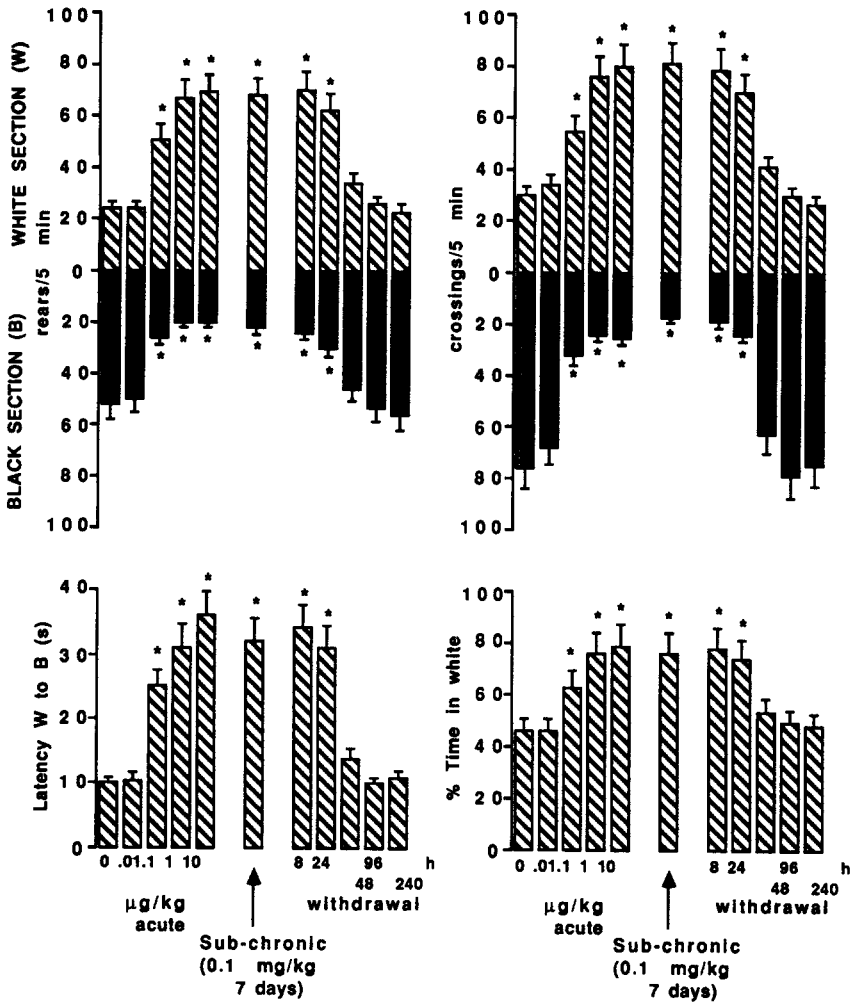

FIG. 2. The effects of ondansetron given acutely, subchronically, or withdrawn from subchronic treatment, in the light/dark exploration test in mouse. Testing was carried out 45 min after acute dosing, 45 min after the first dose on the 7th day of treatment (subchronic), and then at five different times between 8 and 240 hr of withdrawal from subchronic treatment. n = 8 per group. *$p<0.01$ compared to controls. Naive mice were used to obtain each histogram and vehicle control responses were determined on all test days. On no occasion did vehicle control values differ significantly from that presented. Standard errors of the mean are given: for % time spent in the light these were calculated from the original data.

aversion to the light was established and a maximal dose which released the suppressed behaviour selected for administration throughout the 8–240 hr diazepam withdrawal period. In subsequent experiments the increased aversion to light associated with diazepam withdrawal was allowed to develop and at 24-hr treatments with ondansetron or buspirone commenced, or diazepam treatment was reinstated to determine effects on established withdrawal behaviour patterns.

Experiments in Rats

Two series of experiments were carried out to assess rat social interaction. Two strains of rat were used in different components of the study, but there were essentially no strain differences observed. The two strains were male Hooded Lister rats (Glaxo bred, 180–230 g) and male Sprague-Dawley rats (Bradford bred, 225–275 g). Animals were housed in groups of 5 and allowed to adapt to laboratory environments (lights on at 06.00 or 08.00 hr) for at least a week before testing.

The test chambers were wooden (62 × 62 × 33 cm, used for Hooded Lister rats) or Perspex (51 × 51 × 20 cm, used for Sprague-Dawley rats) open-topped boxes. Lighting intensities were 360–380 lux under high light conditions and 3.5 lux under low light conditions.

The behaviours of pairs of rats over 10-minute test periods were observed by remote video recording. Social interaction between the animals was determined by timing (seconds) following with contact, following without contact (Sprague-Dawley rats only), sniffing (including of the hindquarters of Sprague-Dawley rats), crawling under and over, tumbling, boxing and also grooming. Locomotor activity was measured by dividing the arena floor into 9 equal segments and recording the number of lines crossed. Values for time spent in social interaction and moving around the observation cage were determined for individual animals. Naive animals were used in drug-treated groups of six or eight pairs.

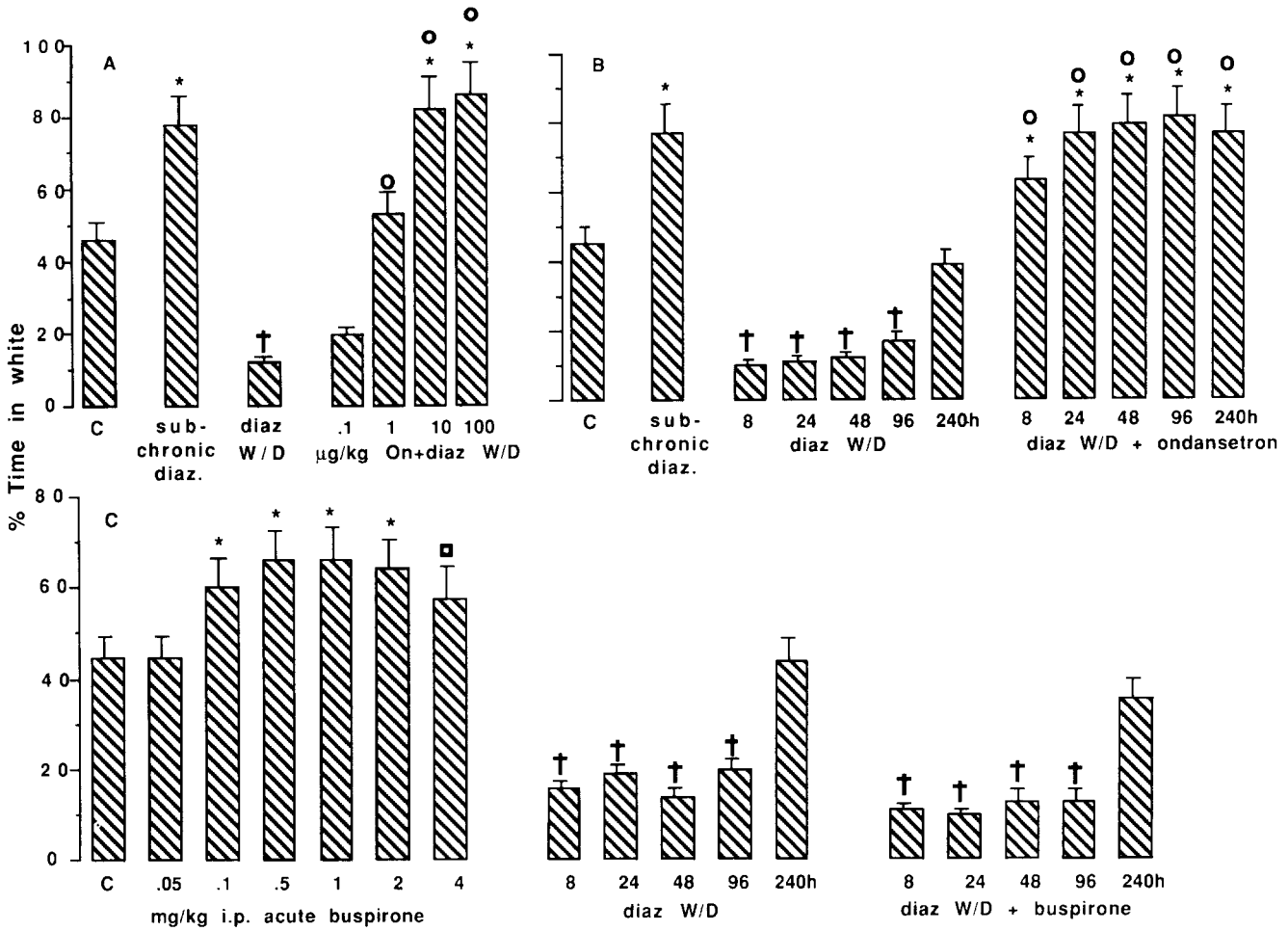

FIG. 3. Assessments in the mouse light/dark exploration test of (A) dose-dependent inhibition by ondansetron (On) or the behavioural consequences of withdrawing (W/D) from subchronic treatment with diazepam (Diaz., 10 mg/kg IP b.i.d. 7 days). Testing was carried out in one group of mice 45 min after the first dose on the 7th day (subchronic diaz.) and then in groups of mice withdrawn for 24 hr from the 7-day treatment, 4 groups receiving 0.1–100 µg/kg IP b.i.d. ondansetron, (B) persistency of the inhibition of diazepam withdrawal effects by ondansetron (10 µg/kg IP b.i.d.). Subchronic diaz. was determined as above, and testing was carried out 8–240 hr following diazepam withdrawal, (C) failure of buspirone (1 mg/kg IP b.i.d.) to inhibit diazepam withdrawal effects. The effects of acute treatments with buspirone are given in addition to the consequences of withdrawing from diazepam (8–240 hr) with and without treatment with buspirone. n = 8 per group. $*p<0.001$ and $\dagger p<0.001$ for increased and decreased exploration in the light respectively (all comparisons to vehicle control, C). $^{\circ}p<0.001$ for inhibition of the decreased exploration in the light by ondansetron. Naive mice were used to obtain each histogram and vehicle control responses were determined on all test days. On no occasion did vehicle control responses differ from those presented. □ Indicates sedation. s.e. means were calculated from original data.

Experimental Designs

Unless otherwise stated, in both the mouse and the rat studies, naive animals were used for each test group and vehicle control data were obtained on each test occasion. Experimenters remained blind to drug treatment throughout the studies.

In Sprague-Dawley rats diazepam and ondansetron were given acutely over wide dose ranges (45 min pretreatment) to establish doses which released the suppressed social interaction. Relatively high doses of diazepam (10 mg/kg IP b.i.d.) and ondansetron (0.1 mg/kg IP b.i.d.) were then given for 7 days followed by abrupt withdrawal of treatment and measurement of effects on rat social interaction at 8, 24, 48 and 96 hr after withdrawal. In subsequent experiments the influence of ondansetron treatment (10 µg/kg IP b.i.d.) was determined on enhancement of the suppressed rat behaviour and weight loss following withdrawal from the subchronic treatment with diazepam.

Tolerance studies were carried out using Hooded Lister rats. The rats were treated orally twice daily with either vehicle (5% acacia), ondansetron (0.01 mg/kg) or diazepam (2 mg/kg). Groups of 8 pairs of rats were tested in the social interaction test under high light conditions after 1, 7, 14 or 21 days of treatment, 45 min after the last dose.

In a second experiment, Hooded Lister rats were treated as above, but with 20 times the doses of ondansetron (0.2 mg/kg) and diazepam (40 mg/kg). After 7 or 21 days of treatment and 12 hr after the last dose, groups of 8 pairs of rats were treated with either vehicle or a test dose (0.01 mg/kg ondansetron or 1.5 mg/kg diazepam) of the appropriate compound. They were tested in the social interactions test 45 min later.

To assess antagonism of the effects of withdrawal from diazepam treatment in Hooded Listar rats, these were treated orally twice daily for 7 days with diazepam (40 mg/kg). Twenty-four hours after the last dose, the rats were treated with the benzodiazepine antagonist flumazenil, 10 mg/kg orally. At the same time, the rats were treated orally with either vehicle, ondansetron (0.01 mg/kg) or diazepam (2 mg/kg). The social interaction of pairs of rats was assessed 45 min later under low light, familiar conditions.

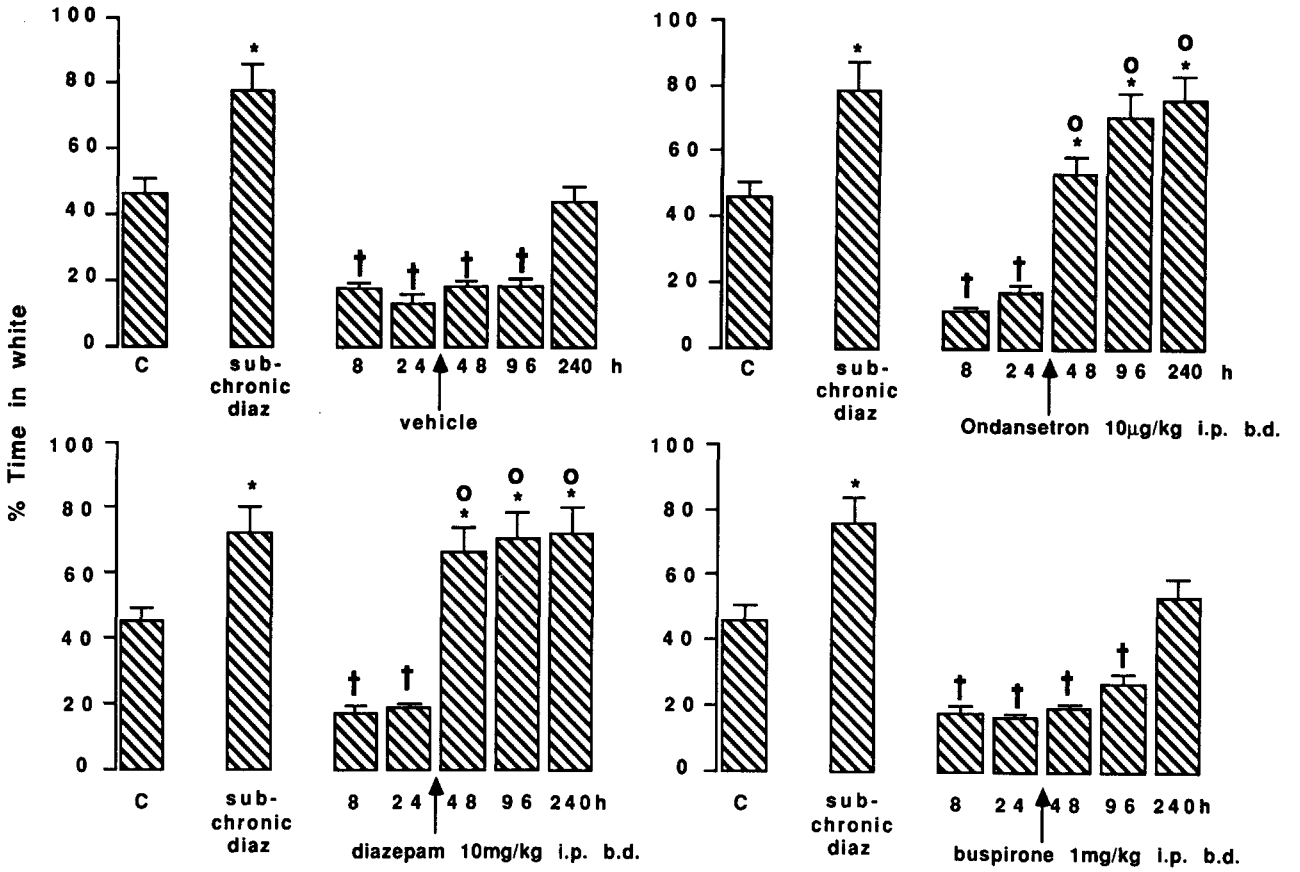

FIG. 4. Abilities of ondansetron (10 μg/kg IP b.i.d.) and diazepam (10 mg/kg IP b.i.d.), but not buspirone (1 mg/kg IP b.i.d.), to inhibit an established reduction in exploration in the light caused by withdrawing from subchronic treatment with diazepam (diaz., 10 mg/kg IP b.i.d. 7 days), measurements being obtained using the light/dark exploration test in mouse. Testing was carried out in groups of mice 45 min after the first dose on the 7th day (subchronic diaz.) and then in groups of mice withdrawn from diazepam for 8–240 hr. Treatment with ondansetron, diazepam, buspirone or vehicle commenced immediately after the 24-hr W/D test. n = 8 per group. *$p<0.001$ and †$p<0.001$ for increased and decreased exploration in the light respectively (all comparisons to vehicle control, C). °$p<0.001$ for inhibition of the decreased exploration in the light. Naive mice were used to obtain each histogram and vehicle control responses were determined on all test days. On no occasion did vehicle control values differ significantly from that presented. Standard errors of the means were calculated from original data.

In a similar test, the effect of flumazenil, 10 mg/kg orally, in rats treated twice daily for 7 days with either 0.2 or 1 mg/kg ondansetron orally, was determined.

Analysis of Results

The results from the light/dark exploration and social interaction tests were analysed by analysis of variance followed by Dunnett's test for multiple comparisons.

Drugs

Ondansetron [(1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one hydrochloride dihydrate, Glaxo Group Research Ltd.] was dissolved in distilled water for IP injection or in 5% acacia for oral administration. Buspirone hydrochloride (Bristol Myers) was dissolved in distilled water. Diazepam (Roche or Evans Medical) for IP injection was dissolved in minimum polyethylene glycol, made up to volume with distilled water; for oral administration, it was suspended in 5% acacia solution. Doses are expressed as the base and were administered in a volume of 1 ml/100 g body weight to mice, 1 ml/kg (IP) or 5 ml/kg (oral) body weight to rats.

RESULTS

The Mouse Light/Dark Test

Acute treatment with diazepam (0.1–1.0 mg/kg IP) or ondansetron (0.1–10 μg/kg IP) reduced aversive behaviour in the mouse light/dark exploration test, as reported previously (11). Since % time spent in the white was shown to reflect the overall change in behavioural profile, this measure was selected for presentation of data in Figs. 3–6.

Figures 1 and 2 show that the reduced aversion for the light caused by acute treatments with diazepam and ondansetron was maintained after 7 days of twice daily high dosage (10 mg/kg IP diazepam, 0.1 mg/kg IP ondansetron). Tolerance to the sedative action of 10 mg/kg diazepam, indicated as a marked reduction of overall behavioural activity in both light and dark compartments, was apparent and previous experiments have shown that this occurs by day 3 (6). Clear differences were seen on withdrawal from subchronic treatments: thus, marked aversion for the light compartment of the test box was seen 8–96 hr after withdrawing from diazepam treatment (control values were regained 240 hr after withdrawal) (Fig. 1), whilst the reduced aversion caused by subchronic ondansetron treatment waned over 24–48 hr following withdrawal, and values then returned to control levels, with no

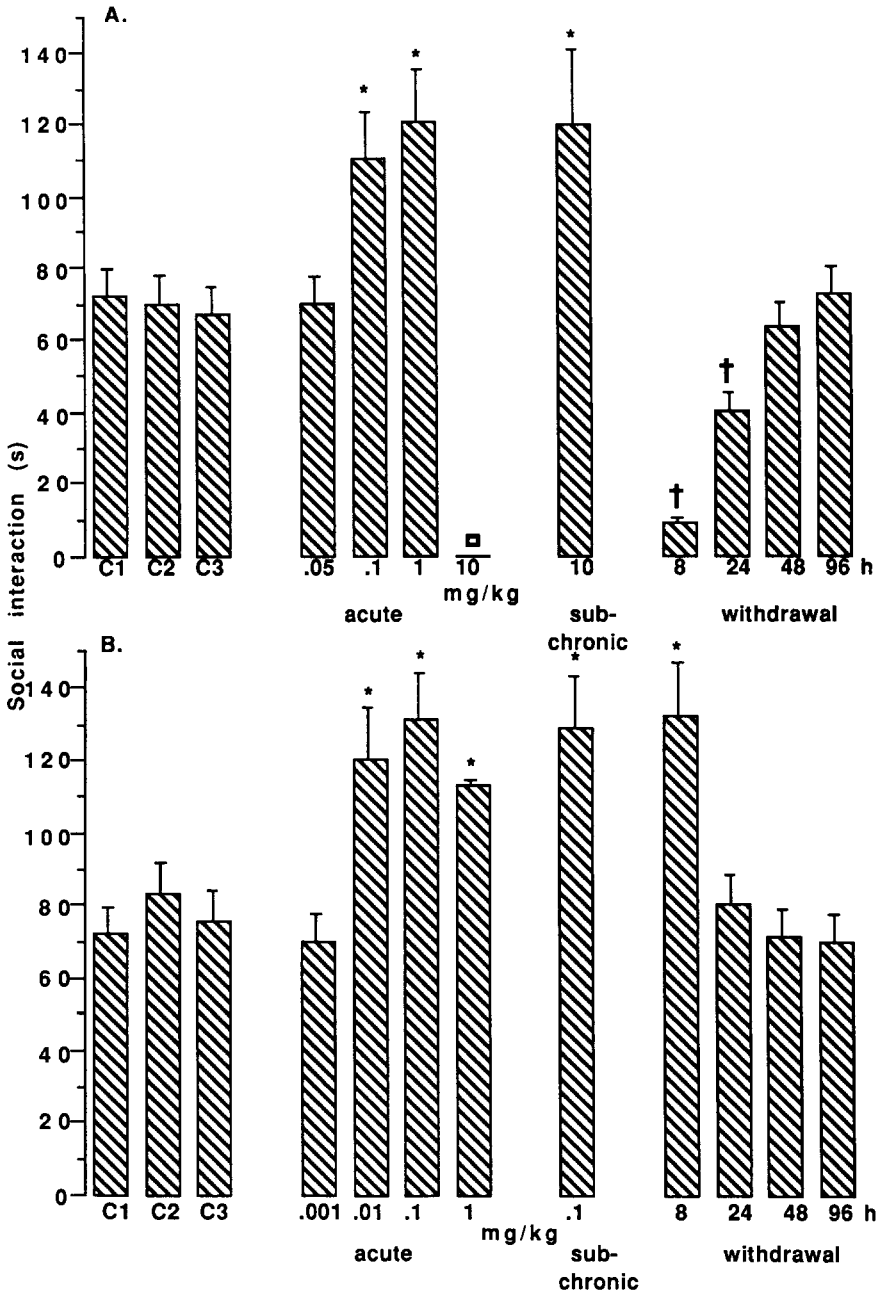

FIG. 5. The effect on rat social interaction of (A) diazepam (doses in mg/kg IP) and (B) ondansetron (doses in μg/kg IP) given acutely (45 min pretreatment), subchronically (test 45 min after administration of the first dose on the 7th day), or withdrawn from the subchronic treatments for 8–96 hr. C1, C2 and C3 represent the vehicle control responses for the acute, subchronic and withdrawal studies. n = 6 pairs per group. *$p<0.001$ for increased social interaction, †$p<0.05$ for decreased social interaction. □ Indicates sedation. s.e. means are given. Locomotor activity remained constant throughout these experiments (71 ± 7.6 to 84 ± 8.7 and 75 ± 8.2 to 89 ± 9.3 crossings/5 min respectively for diazepam and ondansetron) except where sedation developed at 10 mg/kg IP diazepam (18 ± 2.1 crossings/5 min, $p<0.001$).

indication of increased aversion during the 240 hr following withdrawal from 7 days of treatment with high dosage ondansetron (Fig. 2).

Ondansetron, 1–100 μg/kg IP, inhibited the increased aversion of mice for the light compartment of the light-dark box caused by withdrawal from 7 days of treatment with high dosage diazepam (measurements taken at 24 hr following withdrawal from diazepam) (Fig. 3). If ondansetron, 10 μg/kg IP, was given twice daily throughout the period of increased aversion caused by withdrawal from diazepam treatment then not only was the increased aversion prevented, but animals exhibited reduced aversion as seen during the treatment periods with diazepam or ondansetron alone (Fig.

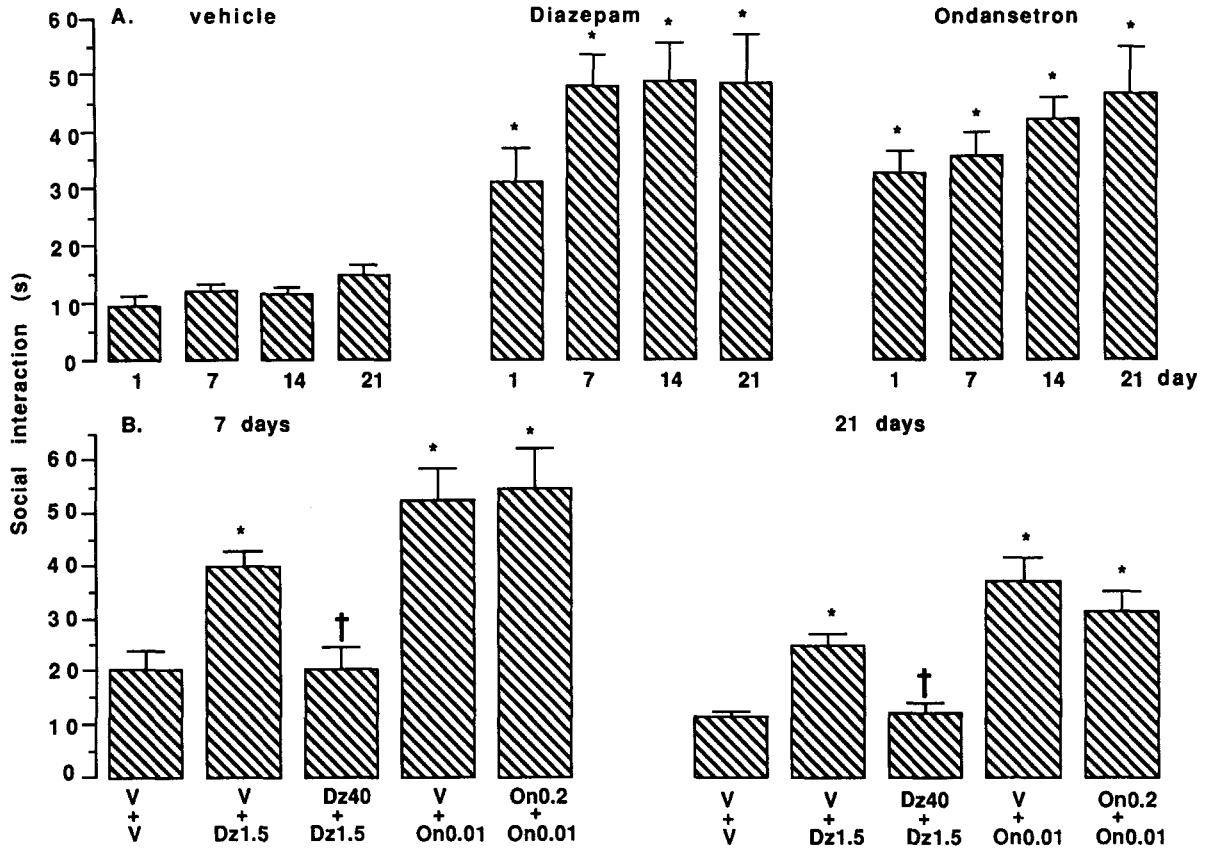

FIG. 6. Use of the rat social interaction test to assess potential for tolerance development to (A) low and (B) high dosage treatment with diazepam or ondansetron. The low dose studies used 2 mg/kg PO b.i.d. diazepam and 0.01 mg/kg PO b.i.d. ondansetron. In these studies $*p<0.05$ compared to control. In the high dose studies diazepam, 1.5 mg/kg PO (Dz 1.5) or ondansetron, 0.01 mg/kg PO (On 0.01) were given after subchronic oral treatment twice daily with vehicle (V), diazepam 40 mg/kg (Dz 40) or ondansetron (On 0.2) for 7 or 21 days. In these studies $*p<0.05$ compared to V + V control, $\dagger p<0.05$ compared to V + Dz 1.5 group. Rats were tested under high light, unfamiliar conditions. n = 8 pairs per group. s.e. means given.

3). In contrast to ondansetron, twice daily dosing with buspirone during the phase of withdrawal from diazepam failed to influence the increased aversion (Fig. 3). The dose of buspirone used was equieffective with the dose of ondansetron against the suppressed behaviour when given acutely. Higher doses of buspirone given subchronically caused sedation which interfered with behaviour, although marked sedation was only apparent following a single challenge with 4 mg/kg IP buspirone (Fig. 3).

In subsequent studies diazepam, 10 mg/kg IP, was given twice daily for 7 days and withdrawn. The increased aversion to the light compartment associated with withdrawal was allowed to develop for 24 hr and then, when the maximum withdrawal effects were established, mice were treated with vehicle, ondansetron or buspirone, or treatment with 10 mg/kg diazepam was reinstated (Fig. 4). In the vehicle-treated mice the withdrawal effects persisted for 96 hr, but in those animals receiving ondansetron or diazepam the withdrawal effects were inhibited at both the 48-hr and 96-hr test times.

In contrast, the responses of the buspirone-treated mice were indistinguishable from those of the withdrawn controls (Fig. 4).

The Rat Social Interaction Test

Assessments of rat social interaction showed that both diazepam (0.1–1 mg/kg IP) and ondansetron (0.01–1 μg/kg IP) reduced the suppressed behaviour or Sprague-Dawley rats placed in an unfamiliar, highly illuminated arena (Fig. 5). These results confirm those previously published (11). The increases in rat social interaction caused by diazepam and ondansetron were maintained on subchronic (7-day, twice daily) treatments using high dosages (10 mg/kg IP diazepam, 0.1 mg/kg IP ondansetron). As in the mouse, the high dose of diazepam caused sedation, but tolerance to this effect developed over the 7-day period (Fig. 5).

Subchronic treatment of Hooded Lister rats with submaximally effective doses of ondansetron (0.01 mg/kg) or diazepam (2 mg/kg) orally for 21 days also failed to induce tolerance to the effects in the social interaction test (Fig. 6). However, when high doses of ondansetron (0.2 mg/kg) and diazepam (40 mg/kg) were administered for 7 or 21 days, there was no tolerance to the effect of a test dose of ondansetron, but tolerance to diazepam was complete, even after only 7 days' treatment (Fig. 6).

Following withdrawal of subchronic diazepam treatment from the Sprague-Dawley rats there was marked suppression of social interaction beyond control values. This effect peaked at 8 hr following withdrawal, but was also significant at 24 hr (Fig. 5). In contrast, withdrawal from the subchronic treatment with ondansetron lead only to a decline, over 24–48 hr, of the reduction in suppressed behaviour to control values (Fig. 5). In Hooded Lister rats withdrawal from diazepam (40 mg/kg IP b.i.d. PO 7 days) was associated with weight loss (mean change −3.8 g, range −7 to −2 g), whilst body weight was maintained in rats withdrawn from ondansetron (0.2 mg/kg PO b.i.d. 7 days) (mean change +0.5 g, range −2 to +4 g) or vehicle (mean change 0 g, range −2 g to +2 g).

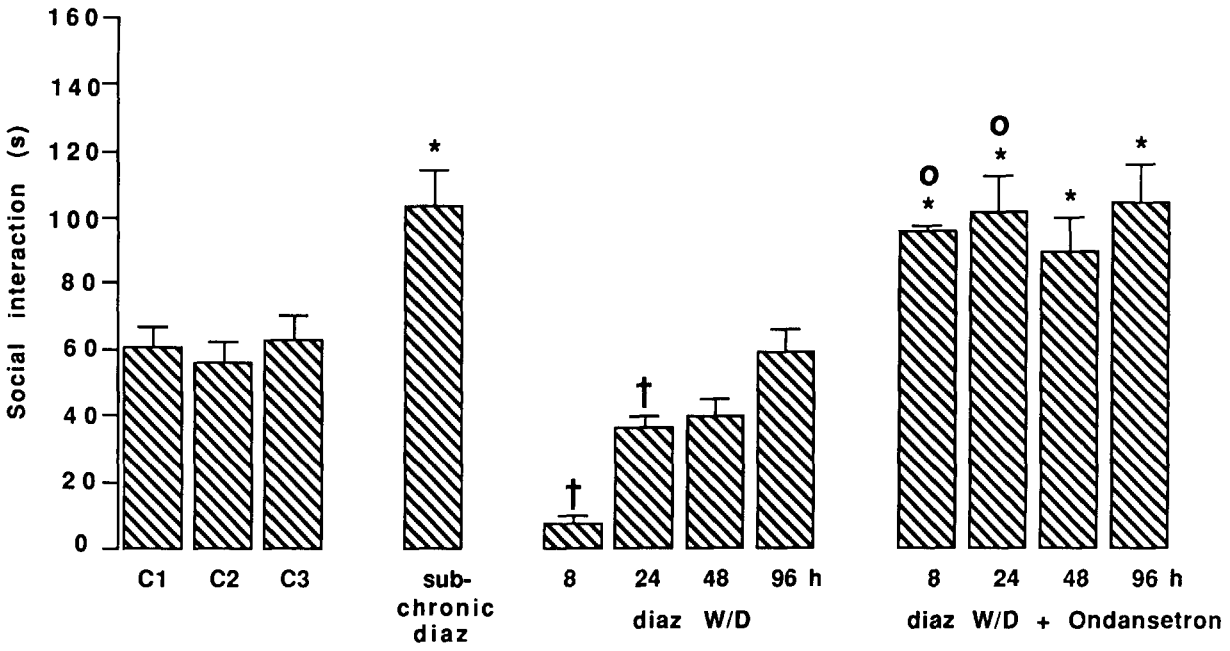

FIG. 7. The effect on social interaction in rats of diazepam (diaz., 10 mg/kg IP b.i.d. 7 days, test 45 min after administration of the first dose on the 7th day) given subchronically, and the effects of withdrawing (W/D) from the subchronic treatment with and without treatment with ondansetron (10 μg/kg IP b.i.d., dosing schedules as described for the mouse). C1, C2 and C3 represent the vehicle control responses for the subchronic diazepam, diazepam withdrawal and diazepam withdrawal/ondansetron studies. n = 6 pairs per group. *$p<0.05$ for increased social interaction, †$p<0.01$ for decreased social interaction, °$p<0.001$ for inhibition of the decreased social interaction by ondansetron. Standard errors of the means are given. Locomotor activity remained constant throughout these experiments (67 ± 7.6 to 81 ± 9.0 crossings/5 min).

In rats withdrawn from diazepam, ondansetron, 10 μg/kg IP twice daily, released the suppressed social interaction (Fig. 7). And, as in the mouse, ondansetron not only inhibited the suppressed behaviour associated with withdrawal, but actually increased social interaction so that it was significantly higher than control values (Fig. 7). The weight loss in diazepam-treated rats was also prevented by ondansetron.

A decrease in social interaction could be precipitated by flumazenil in Hooded Lister rats which had been treated with 40 mg/kg diazepam for 7 days and tested under low light, familiar conditions (Fig. 8). Flumazenil did not cause any behavioural effects in rats subchronically treated with 0.2 or 1.0 mg/kg ondansetron and tested under the same conditions (Fig. 8). The decrease in social interaction induced by flumazenil in diazepam-treated rats was antagonised by a further dose of diazepam (2 mg/kg) and also by ondansetron (0.01 mg/kg) (Fig. 8).

DISCUSSION

The disinhibitory effects of ondansetron in the present study in the mouse and rat confirm previous observations (11,14). The effect of ondansetron in the social interaction test is clearly robust and could be shown in both Sprague-Dawley and Hooded Lister strains of rat. Furthermore, the exclusion or inclusion of certain behaviours, such as genital sniffing or following without contact, appear irrelevant to the ability of this drug to release suppressed behaviour. The same conclusions can also be drawn for diazepam and buspirone.

During chronic treatment with ondansetron in either the rat or mouse using various dosing regimens there was no evidence of tolerance to the disinhibitory activity which was sustained throughout the dosing period. Similar data were obtained for diazepam at low doses, but at high doses clear tolerance developed to both the disinhibitory and sedative properties, supporting the data obtained by File (8). Even at lower doses, tolerance to the sedative effect developed within 3 to 4 days.

The development of tolerance to a drug and the emergence of a withdrawal syndrome upon cessation of treatment implies that an adaptive cellular change has occurred. In man, there is little doubt that tolerance develops to the sedative actions of benzodiazepines and that abrupt cessation of treatment leads to an adverse and clearly recognisable syndrome (12). However, in rodents, these withdrawal phenomena have not been easy to demonstrate. Recently, File and colleagues (7) showed an increase in aversive behaviour in the plus-maze in rats following withdrawal from subchronic chlordiazepoxide treatment. In the present study, withdrawal from chronic diazepam treatment in the mouse resulted in a clear behavioural change characterised by a marked suppression of behaviour to a level where the mice appeared to find the light stimulus more aversive than control mice. In the social interaction test there was a clear strain difference. Withdrawn Sprague-Dawley rats showed suppressed behaviour under high light/unfamiliar conditions within 8 hr of cessation of dosing with diazepam. However, Hooded Lister rats, even under low light/familiar conditions, failed to show any spontaneous changes in behaviour, but required flumazenil to precipitate a withdrawal syndrome. This strain difference was not due to differences in dose of diazepam (unpublished). In neither species, either with or without flumazenil, did withdrawal from subchronic treatment with ondansetron result in a change in behaviour which could be described as a 'withdrawal' effect. Instead, the disinhibitory effect of ondansetron gradually declined until social interaction returned to control levels within 24 to 48 hr. While these latter data are insufficient to show a lack of dependence liability of ondansetron, they suggest that treatment with this drug is unlikely to lead to the kind of problems associated with the persistent use of benzodiazepines.

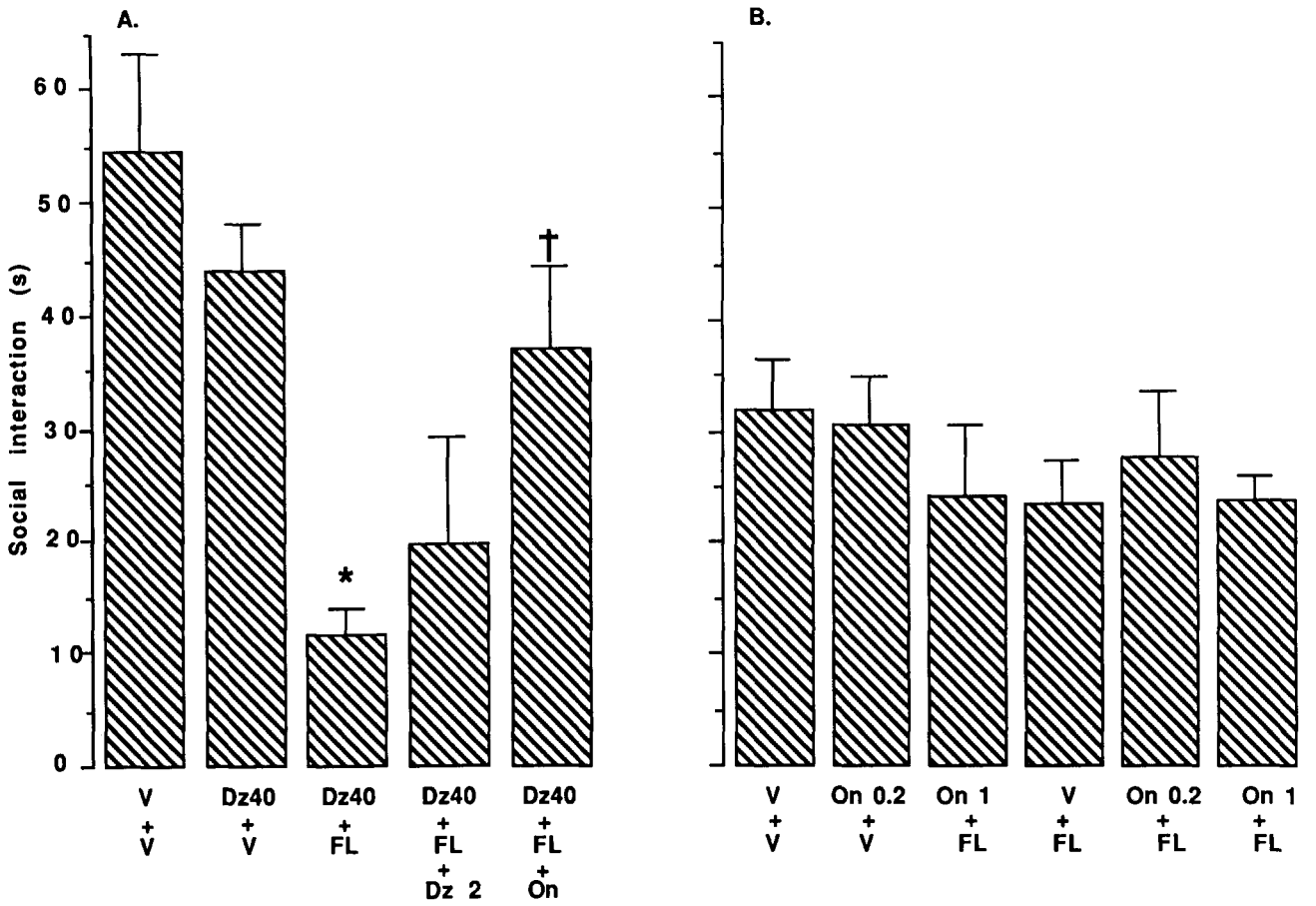

FIG. 8. Influence on rat social interaction of diazepam given subchronically (Dz 40, 40 mg/kg PO b.i.d. 7 days), reduction in the social interaction caused by flumazenil, 10 mg/kg (FL), and inhibition of the flumazenil response by diazepam 2 mg/kg (Dz 2) or ondansetron 0.01 mg/kg (On). All drugs were given PO. *$p<0.05$ compared to the Dz 40/V group, †$p<0.05$ compared to the Dz 40/FL group. (B) The effect of flumazenil, 10 mg/kg PO (FL) on the social interaction of rats treated PO b.i.d. for 7 days with vehicle (V), ondansetron 0.2 mg/kg (On 0.2) or ondansetron 1 mg/kg (On 1). Rats were tested under low light, familiar conditions. n = 8 pairs per group. s.e. means given.

Studies in laboratory animals suggest that ondansetron and other 5-HT_3 receptor antagonists may possess anxiolytic properties in man (11,17). One potential problem is conducting clinical trials in anxious patients is that many will recently have undergone treatment with benzodiazepines; there are also those patients who, to varying degrees, are addicted to them. Such problems have been encountered with the 5-HT_{1A} agonist anxiolytic, buspirone, for which it has been found particularly difficult to treat patients with previous benzodiazepine experience (16). For ondansetron to be of value in the treatment of anxiety disorders, its interaction with benzodiazepines is of fundamental importance. The present studies were carried out to address this question.

The abrupt increase in behavioural suppression seen in mice and rats following cessation of diazepam treatment could clearly be prevented or reversed by ondansetron in low doses. In contrast, buspirone, at equieffective disinhibitory doses, failed to reverse the diazepam withdrawal phenomena. If these data are reflecting the clinical problem found with buspirone then it is unlikely that the same problems will be found for ondansetron. But the data also imply that ondansetron may be useful in helping patients addicted to benzodiazepines by alleviating the withdrawal syndrome.

Reversal of the withdrawal effects from diazepam by ondansetron could be interpreted as being simply the disinhibitory effects of ondansetron superimposed on the withdrawal changes. However, if this was the case then buspirone should have produced the same effect. Furthermore, in the rat, 5-HT_3 receptor antagonists do not enhance social interaction under low light conditions in rats familiar with the test arena. However, the most convincing evidence to suggest that reversal of withdrawal-induced suppressed behaviours by ondansetron is not just a manifestation of its disinhibitory action is that the weight loss in rats induced by cessation of diazepam treatment is also prevented.

Other studies have shown that ondansetron is able to prevent the behavioural suppression which occurs after withdrawal from chronic treatment with nicotine, ethanol or cocaine (5) and can reduce ethanol consumption in ethanol-preferring marmosets (13) or rats (15). These properties suggest that ondansetron may influence reward systems such as the mesolimbic dopaminergic pathway (1). Indeed, several studies have shown that dopamine metabolism in terminal areas induced by direct stimulation of this pathway via the ventral tegmental area can be inhibited by 5-HT_3 receptor antagonists [(9,10), Marsden and colleagues personal comunication]. In addition, ICS 205–930 and MDL 72222 can reverse the rewarding effects of acute morphine, nicotine or amphetamine administration in place preference conditioning (3,4).

In conclusion, the present study supports previous observations that 5-HT_3 receptor antagonists can suppress the withdrawal behavioural effects which follow cessation of subchronic treatment with diazepam and other drugs of abuse. Evidence is growing that these effects are different to those that release suppressed behaviour, and may influence reward through an action on the ventral tegmental-mesolimbic system.

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