Pharmacology Biochemistry & Behavior, Vol. 36, pp. 97–104. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Sites of Action of Ondansetron to Inhibit Withdrawal From Drugs of Abuse

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Received 12 January 1990

COSTALL, B., B. J. JONES, M. E. KELLY, R. J. NAYLOR, E. S. ONAIVI AND M. B. TYERS. *Sites of action of ondansetron to inhibit withdrawal from drugs of abuse*. PHARMACOL BIOCHEM BEHAV **36**(1) 97–104, 1990.—The cerebral site of action of the selective 5-HT_3 receptor antagonist ondansetron to influence the behavioural consequences of withdrawal from subchronic treatment with diazepam, ethanol, nicotine or cocaine was studied in the light/dark exploration test in the mouse. The aversive response to the light compartment of the test box was reduced during a subchronic treatment with peripherally administered diazepam, ethanol, nicotine and cocaine, but was exacerbated following withdrawal from the 4 treatments. The behavioural consequences of withdrawal from diazepam (10 mg/kg IP b.i.d. 14 days), ethanol (8%/w/v drinking water for 14 days), nicotine (0.1 mg/kg IP b.i.d. 14 days) or cocaine (1.0 mg/kg IP b.i.d. 14 days) were antagonised by ondansetron injected into the amygdala and dorsal raphe nucleus (1–10 ng); injections of ondansetron (10 ng) into the median raphe nucleus, the nucleus accumbens and striatum were ineffective. It is concluded that the amygdala and dorsal raphe nucleus may be sites of action for ondansetron to antagonise the aversive behaviour caused by withdrawal from 4 common drugs of abuse in a mouse model, and that 5-HT projections from the dorsal raphe nucleus may be involved in aversive behaviour.

Ondansetron 5-HT_3 receptor Intracerebral injection Mouse aversive behaviour Dorsal raphe nucleus Amygdala Drugs of abuse Withdrawal phenomena

THE peripheral administration of the selective 5-HT_3 receptor antagonist ondansetron releases suppressed behaviour in the light/dark exploration test in the mouse, the social interaction test in the rat, and in behavioural tests in the marmoset and cynomolgus monkey (21). It was suggested that the ability of ondansetron to reduce aversive behaviour is predictive of anxiolytic activity, and supports an involvement of 5-hydroxytryptamine (5-HT) in the processes underlying anxiety (7, 17, 20, 30). Localisation of the sites of action of ondansetron could provide important clues in elucidating the 5-HT pathways involved in the aetiology of anxiety or aversive behaviour. 5-HT_3 receptors have recently been identified in limbic and cortical areas of rodent and human brain (3, 4, 22), and it is known that injections of ondansetron, other 5-HT_3 receptor antagonists and diazepam into the amygdala of the mouse brain attenuate aversive responding in the light/dark exploration test (11). A similar response was obtained following the injection of ondansetron into the dorsal raphe nuclei, emphasising the importance of the limbic 5-HT projection.

In addition to reducing aversive behaviour, the peripheral administration of ondansetron has been shown to prevent the aversive responding induced by the withdrawal from drugs of abuse, diazepam, ethanol, nicotine or cocaine (8,9). The site of action of ondansetron to inhibit the behavioural consequence of drug withdrawal remains uncertain. In the present study, using the intracerebral injection technique and the mouse light/dark exploration test, we have investigated the site(s) of action of ondansetron to inhibit the behavioural consequences of withdrawing from treatment with drugs of abuse.

METHOD

The studies used male BKW mice (25–30 g) housed in groups of 10 in conditions of constant temperature (22 ± 1.0°C) and controlled lighting (dark period 07.00–19.00 hr) and fed ad lib on a standard laboratory chow.

Behavioural Experiments

Mice were taken from the dark holding room at 08.30 hr in a dark container to a dimly illuminated room (red illumination) where, after a 1-hr period of adaptation to the new environment, they were placed into the centre of the light area of the test box. The box (45 × 27 × 27 cm high) was open-topped and the base lined into 9-cm squares, two-fifths painted black and illuminated under a dim red light (1 × 60 w, zero lux) and partitioned from the remainder of the box which was painted white and brightly illuminated (1 × 60 w, 400 lux, the red and white light sources 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. The mice were observed by remote videorecording over a 5-min period and four measurements were made (a) the time spent in the white and black sections, (b) the

number of exploratory rearings (lifting of the head with or without lifting of the forelimbs from the floor) in the white and black areas, (c) the number of line crossings (movements of both the front and hind limbs across a line) in the white and black sections and (d) the latency of the initial movement from the white to the black area.

Stereotaxic Implantation of Cannulae and Intracerebral Drug Injection

Mice were anaesthetised with chloral hydrate, 450 mg/kg IP, and placed into the Kopf stereotaxic frame. Using standard stereotaxic techniques, hole(s) were drilled at the point of guide cannula(e) penetration of the skull, guide cannulae were lowered and dental acrylic cement mixed with cyanoacrylate glue was used to bond the perspex guide cannula unit to the skull. The wound was sealed and stylet(s) placed into the cannula(e) which extended 0.5 mm below the bottom of the guide. Prior to intracerebral injection the mice were manually restrained in a soft cloth and after removing the stylet(s) an injection unit(s), connected by pp25 polythene tubing to 5 μl Hamilton syringes, was inserted into the guide and the drug or vehicle solution administered over a 5-sec period, the unit(s) remaining in position for a further 55 sec. Drug solution (0.25 μl) or vehicle was injected bilaterally into the nucleus accumbens (ACB), the amygdala (AMG) or centrally into the dorsal or median raphe nuclei (DRN, MRN); a 0.5 μl volume was injected bilaterally into the caudate-putamen (CP). The injection unit(s) were then withdrawn, the stylet(s) replaced, and the animal placed into the test box for the behavioural measurements.

The guide cannulae were constructed from 6 mm lengths of stainless steel tubing (0.65 mm external diameter) inserted and bonded (with Araldite Epoxy Resin) into predrilled (No. 73 drill) perspex sheets of 3 mm depth. The perspex was also drilled and tapped to allow attachment to the tool carrier of the stereotaxic instrument. The stainless steel tubing extended 3 mm below the base of the perspex and some 1.5 mm into brain tissue. The small size of the perspex blocks (approximately 5.0 mm deep and 5.0 to 8.0 mm wide) ensured a nonintrusive presence on implantation.

The coordinates and lengths of injection unit made from stainless steel tubing (0.3 mm external diameter) were selected such that the drug or vehicle would be injected into the 'centre' of the area under investigation. Cannulae were implanted vertically for injections into the ACB (Ant. 4.5, Vert. 4.2, Lat. ±1.0), the CP (Ant. 3.0, Vert. 3.8, Lat. ±2.3) and central nucleus of the amygdala AMG (Ant. 2.5, Vert. 4.8, Lat. ±2.8) and angled at 30° posterior for injection into the MRN (Ant. −0.5, Vert. −5.4, Lat. 0.0) and DRN [Ant. −0.5, Vert. −3.1, Lat. 0.0; Atlas of Slotnick and Leonard (29)].

Experimental Design

Cannulated mice received subchronic treatments for 14 days with (a) diazepam (10 mg/kg IP b.i.d.), (b) ethanol (8%/w/v in the drinking water); measurement of group intake of fluid indicated an average intake of 7.5 g/kg/24 hr ethanol, (c) nicotine (0.1 mg/kg IP b.i.d.), (d) cocaine (1.0 mg/kg IP b.i.d.) or (e) vehicle IP b.i.d. and were tested during and following drug treatment/intracerebral injection. The dose regimens were selected on the basis of previous studies where acute or chronic treatments were shown to attenuate an aversive response in the mouse model and exacerbate the response following withdrawal, in the absence of gross changes in motor behaviour, e.g., tremor and seizures [see Costall *et al.* (10,12); Barry *et al.* (5)]. Control data were obtained using noncannulated untreated mice and cannulated mice receiving intracerebral injection of vehicle (saline). Animals were used in treatment groups of 5 and treatment regimes were randomised between test periods. No significant differences were recorded between the control values obtained in different test periods or days. It is emphasised that animals were used on a single occasion only and all cannulated mice were subsequently killed and the site(s) of drug/vehicle deposition identified from the site of the injection cannula track in frozen sections.

Drugs

Ondansetron (GR38032F) (1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-l-yl)-methyl]-4H-carbazol-4-one, $HCl{\cdot}2H_2O$, Glaxo Group Research Ltd.), ethanol (J. Burroughs Ltd.), nicotine hydrogen tartrate (B.D.H.) and cocaine·HCl (B.D.H.) were freshly prepared in distilled water and diazepam (Roche Products Ltd.) was dissolved in the minimum quantity of polyethylene glycol and prepared to volume with distilled water. Doses are expressed as the base and when administered intraperitoneally were given in a volume of 1 ml/100 g body weight.

RESULTS

General Observations

The illumination and relative size of the two compartments of the test box ensured that mice, which had not been subject to stereotaxic surgery or drug treatment, moved from the white to the black section within 10 to 12 sec and spent approximately equal time in each section. The number of line crossings in the two sections was similar, although rearing behaviour was greater in the black (41 ± 5/5 min, as compared to the white section (23 ± 3.1/5 min) (Fig. 1). Cannulated mice demonstrated a similar exploratory profile to noncannulated mice in the black and white test box, and thus appeared to be unencumbered by the cannulae implantation.

In all experiments measurements were made of the latency of movement of mice into the black section, the % time spent in each compartment and the number of rears and line crossings per 5-min period. Modifications to all such parameters are reported on Fig. 1 for ondansetron. As can be seen for this agent, increases or decreases in the incidence of rears or line crossings in one section of the box corresponded respectively to decreases or increases in these behaviours in the second section of the test box. The same correspondence was seen in all subsequent drug treatments and interactions. For conciseness of presentation, only the latency of initial movement into the black section and % time spent in the two sections is reported on the subsequent figures. Withdrawals from chronic treatment with diazepam, ethanol, nicotine or cocaine were not associated with incoordinated motor behaviour, seizures or tremors.

The Effects of Discrete Ondansetron Injections on Mouse Exploratory Behaviour

The injection of ondansetron (0.01–1.0 ng) into the dorsal raphe nucleus or amygdala increased the time that mice spent in the white area and delayed their movement from the white to the dark section. The increases in time spent in the white area were associated with increases in the number of rearings and line crossings with corresponding decreases for each of these parameters in the black section. Such drug-induced changes in rearing and line crossings in the black section were considerably in excess of those caused by the injection of vehicle. The injection of ondansetron (10 ng) into the median raphe nucleus caused a similar change in exploratory behaviour: lower doses (0.01 to 1.0 ng) were ineffective. The injection of ondansetron into the nucleus accumbens and caudate putamen failed to modify the exploratory behaviour of mice (Fig. 1).

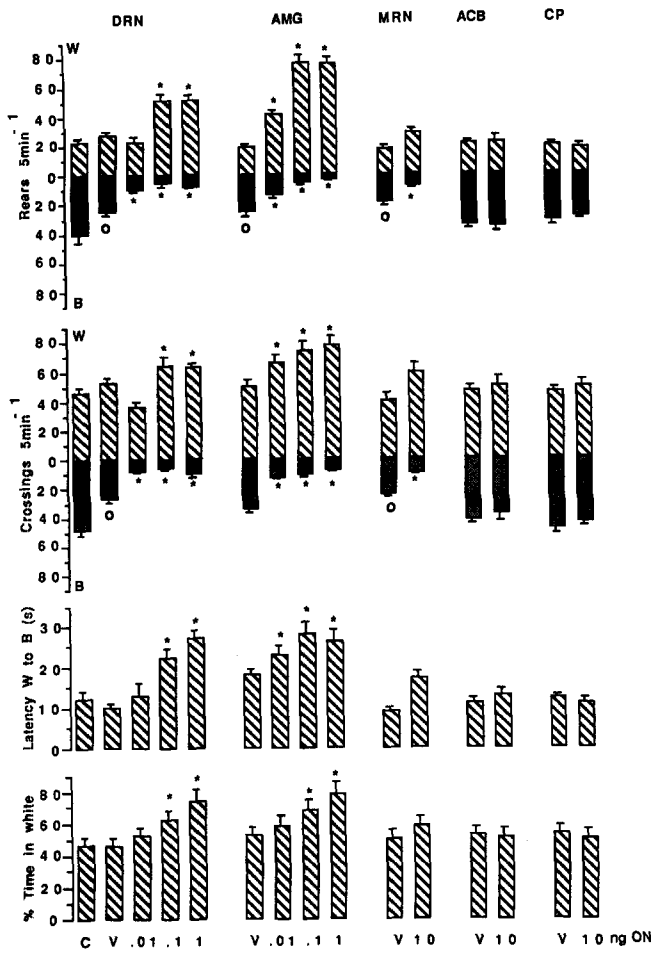

FIG. 1. Effects of ondansetron (ON) following injection into the dorsal and median raphe nucleus (DRN, MRN), the amygdala (AMG), the nucleus accumbens (ACB) and caudate-putamen (CP) on mouse rearing behaviour, line crossings, the % time spent in and initial movement between the white (W) and black (B) sections of a box separated into light (white illumination) and dark (red illumination) compartments connected by an opening located at floor level. Each column is the mean of 10 determinations with s.e. mean shown by vertical bar (calculated from original data for % time in white). Significant increases or decreases in responding compared with the vehicle (V) controls (C is the response of noncannulated nontreated mice) are indicated as $*p<0.05$–$p<0.001$; significant reductions in responding in vehicle-treated animals as compared to C are indicated as $^{o}p<0.05$ (one-way ANOVA followed by Dunnett's t-test).

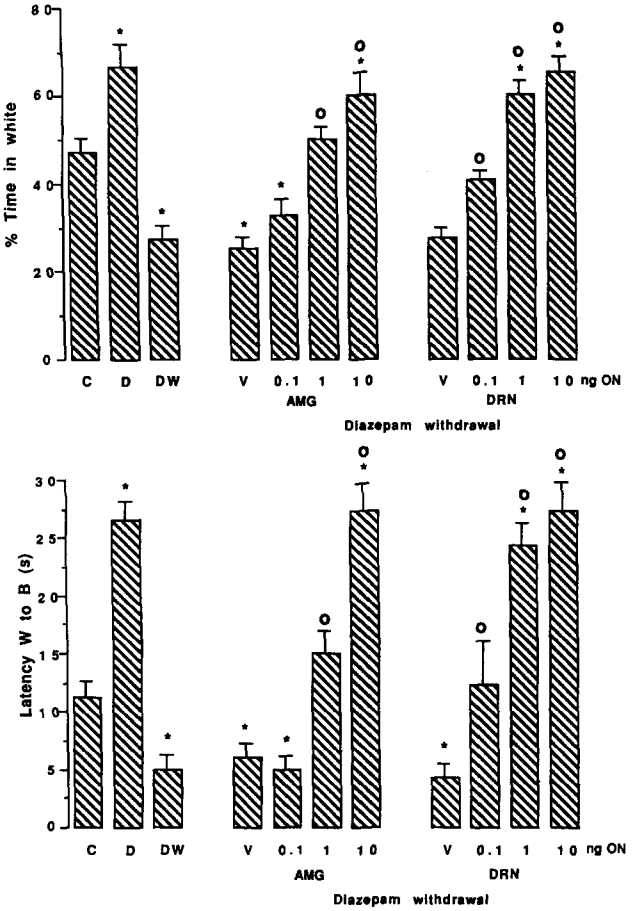

FIG. 2. The effects of ondansetron (ON) injected into the dorsal raphe nucleus (DRN) and amygdala (AMG) to inhibit the reduced exploration in the light caused by withdrawal from a subchronic treatment with diazepam in the mouse. Mice were injected daily with diazepam (10 mg/kg IP b.i.d.) and tested on the 7th day of treatment (D) and 48 hr following withdrawal from a 14-day administration (DW). Each column is the mean of 10 determinations with s.e. mean shown by a vertical bar (calculated from original data for % time in white). Significant increase or decrease in the % time spent in the white section and latency of initial movement from the white (W) to the black section (B) of the test box compared to C (nontreated cannulated mice) are indicated as $*p<0.05$–$p<0.001$ reversal of the effects of DW by treatment with ON is indicated as $^{o}p<0.01$–$p<0.001$ (one-way ANOVA followed by Dunnett's t-test).

The Effects of Ondansetron on the Behavioural Consequences of Withdrawal From Subchronic Treatment With Diazepam, Ethanol, Nicotine or Cocaine

Mice treated with diazepam (10 mg/kg IP b.i.d.) for 7 days showed a delayed response in moving from the white to the black section, and an increased time spent in the white section. Forty-eight hr following withdrawal from a 14-day treatment with diazepam, the profile was reversed, mice showed a reduced latency in moving into the black section and a reduced time in the white section. In mice cannulated to allow injections into the amygdala or dorsal raphe nucleus, the injection of vehicle failed to modify these responses following cessation of diazepam treatment. The injection of ondansetron into the amygdala and dorsal raphe nucleus (1–10 ng and 0.1–10 ng respectively) prevented the development of the behavioural changes observed after 48-hr withdrawal from diazepam. The latency and time spent in the white were comparable to those obtained in mice on 7 days of continuing treatment with diazepam (Fig. 2). The high dose of ondansetron (10 ng) injected into the medial raphe nucleus, caudate putamen or nucleus accumbens failed to prevent the behavioural changes observed after 48-hr withdrawal from diazepam (Fig. 3).

Mice receiving 8%/w/v ethanol in the drinking water for 7 days showed a delayed response in moving from the white to the black section on day 7 and an increased time spent in the white section. Forty-eight hr following withdrawal from a 14-day treatment with ethanol, mice showed a reverse profile, moving with a reduced latency into the black section where the mice spent more time. The

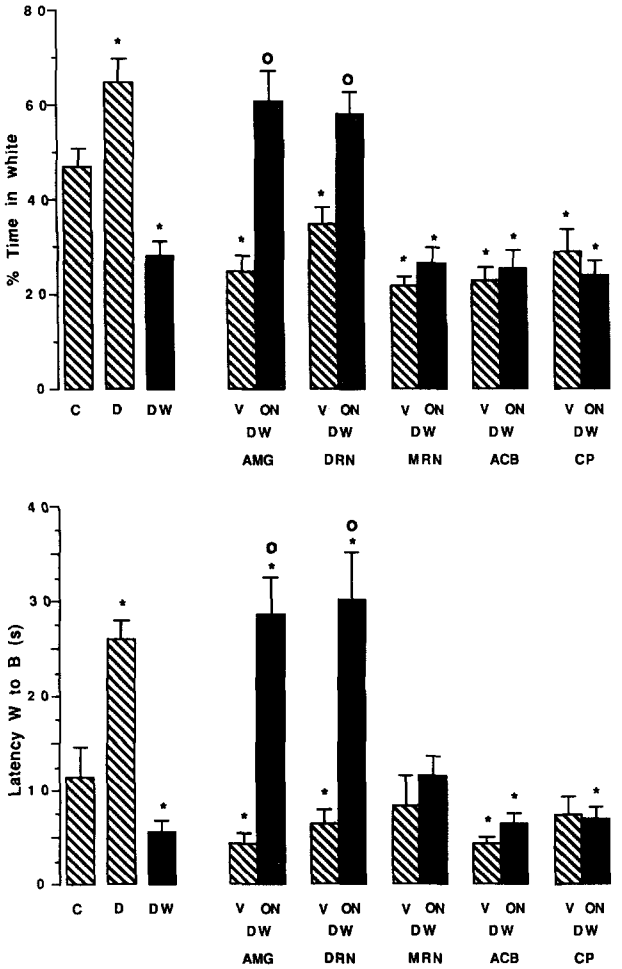

FIG. 3. The effects of ondansetron (ON, 10 ng) injected into the amygdala (AMG), dorsal and median raphe nucleus (DRN, MRN), nucleus accumbens (ACB) or caudate-putamen (CP) to inhibit reduced exploration in the light caused by the withdrawal from subchronic treatment with diazepam in the mouse. Mice were injected daily with diazepam (10 mg/kg IP b.i.d.) and tested on the 7th day of treatment (D) and 48 hr following withdrawal from a 14-day administration (DW). Each column is the mean of 10 determinations with s.e. mean shown by a vertical bar (calculated from original data for % time in white). V is the response of mice receiving intracerebral injection of vehicle. Significant increases or decreases in the % time spent in the white section and latency of initial movement from the white (W) to the black (B) section of the test box compared to C (nontreated cannulated mice) are indicated as $*p<0.05$–$p<0.001$, reversal of the effects of DW by treatment with ON are indicated $^{o}p<0.001$ (one-way ANOVA followed by Dunnett's *t*-test).

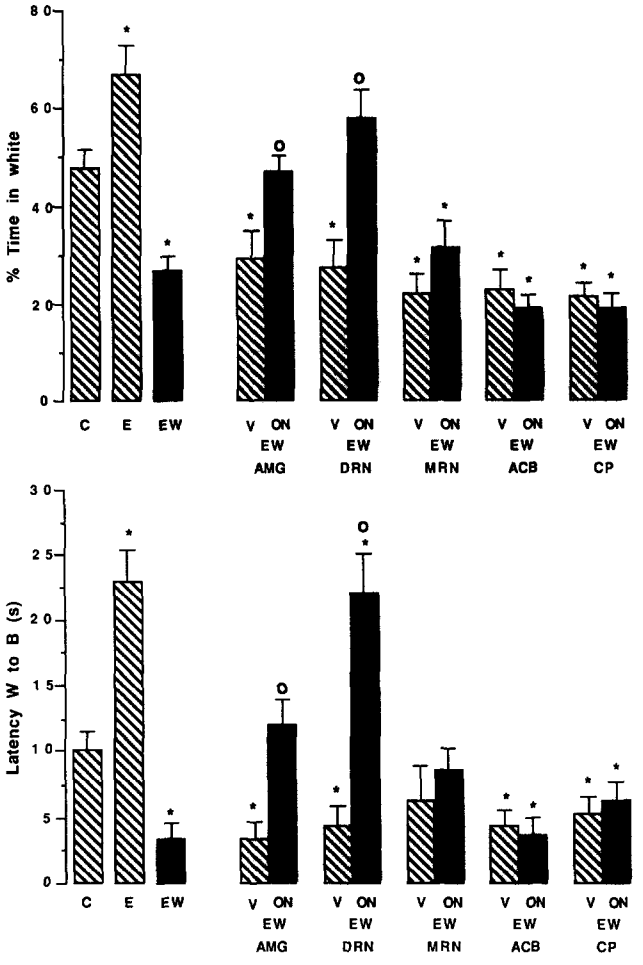

FIG. 4. The effects of ondansetron (ON, 10 ng) injected into the amygdala (AMG), dorsal and median raphe nucleus (DRN, MRN), nucleus accumbens (ACB) or caudate-putamen (CP) to inhibit reduced exploration in the light caused by the withdrawal from subchronic treatment with ethanol in the mouse. Mice received ethanol 8%/w/v in the drinking water and were tested on the 7th day of treatment (E) and 48 hr following withdrawal from a 14-day administration (EW). Each column is the mean of 10 determinations with s.e. mean shown by a vertical bar (calculated from original data for % time in white). V is the response of mice receiving intracerebral injection of vehicle. Significant increases or decreases in the % time spent in the white section and latency of initial movement from the white (W) to the black (B) section of the test box compared to C (nontreated cannulated mice) are indicated as $*p<0.01$–$p<0.001$, reversal of the effects of EW by treatment with ON are indicated as $^{o}p<0.01$–$p<0.001$ (one-way ANOVA followed by Dunnett's *t*-test).

injection of ondansetron (10 ng) into the amygdala inhibited the consequences of withdrawal from ethanol, values returning to those of nontreated control rats. Similar results were obtained following the injection of ondansetron (10 ng) into the dorsal raphe nucleus, with a trend for values to approach those shown by mice receiving the 7-day treatment with ethanol (Fig. 4). Ondansetron (10 ng) injected into the medial raphe nucleus, the caudate putamen or nucleus accumbens failed to prevent the behavioural changes observed after 48-hr withdrawal from ethanol treatment (Fig. 4).

Mice receiving nicotine (1.0 mg/kg b.i.d. IP) showed a delayed response in moving from the white to the black section on day 14 and an increased time spent in the white section. Forty-eight hr following withdrawal from a 14-day treatment with nicotine, mice showed a reverse profile, moving with a reduced latency into the black section where they spent more time. The injection of ondansetron (10 ng) into the amygdala or dorsal raphe nucleus prevented these effects, and data for mice injected with ondansetron into the dorsal raphe nucleus were similar to those for mice on nicotine (Fig. 5). Ondansetron (10 ng) injected into the medial raphe nucleus, caudate putamen or nucleus accumbens failed to prevent the behavioural changes observed after 48-hr withdrawal from nicotine (Fig. 5).

Mice receiving cocaine (1.0 mg/kg b.i.d. IP) for 14 days

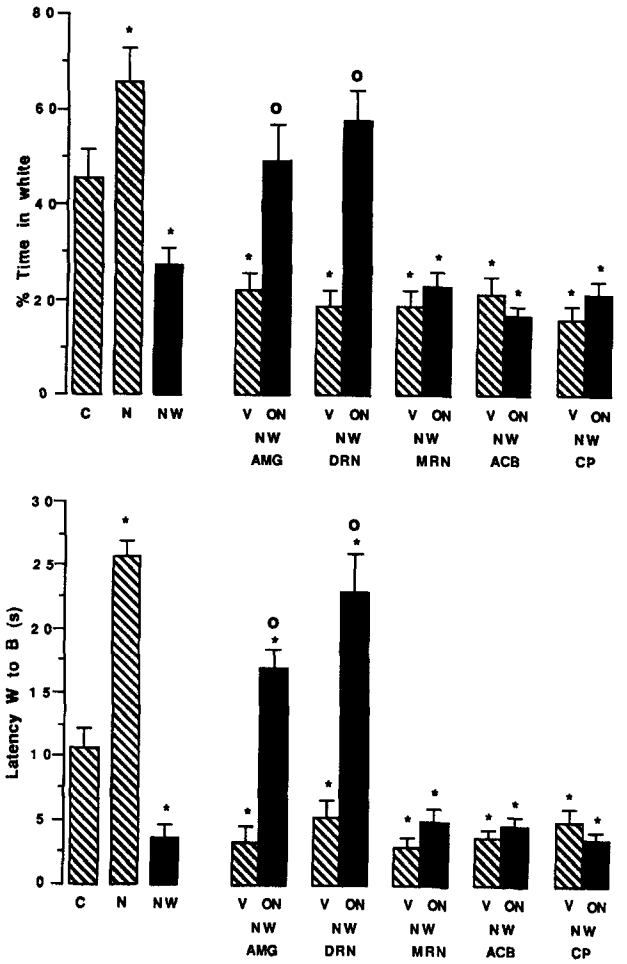

FIG. 5. The effects of ondansetron (ON, 10 ng) injected into the amygdala (AMG), dorsal and median raphe nucleus (DRN, MRN), nucleus accumbens (ACB) or caudate-putamen (CP) to inhibit reduced exploration in the light caused by the withdrawal from subchronic treatment with nicotine in the mouse. Mice were injected daily with nicotine (0.1 mg/kg IP b.i.d.) and tested on the 14th day of treatment (N) and 48 hr following withdrawal from the 14-day administration (NW). Each column is the mean of 10 determinations with s.e. mean shown by a vertical bar (calculated from original data for % time in white). V is the response of mice receiving intracerebral injection of vehicle. Significant increases or decreases in the % time spent in the white section and latency of initial movement from the white (W) to the black (B) section of the test box compared to C (nontreated cannulated mice) are indicated $*p<0.05$–$p<0.001$, reversal of the effects of NW by treatment with ON are indicated $^{o}p<0.01$–$p<0.001$ (one-way ANOVA followed by Dunnett's t-test).

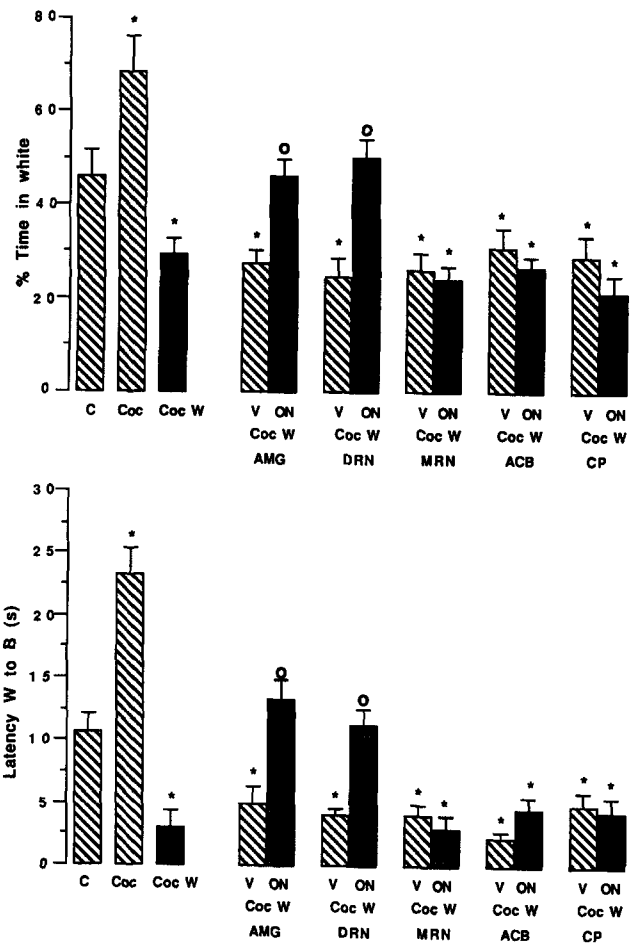

FIG. 6. The effects of ondansetron (ON, 10 ng) injected into the amygdala (AMG), dorsal and median raphe nucleus (DRN, MRN), nucleus accumbens (ACB) or caudate-putamen (CP) to inhibit reduced exploration in the light caused by the withdrawal from subchronic treatment with cocaine in the mouse. Mice were injected daily with cocaine (1.0 mg/kg IP b.i.d.) and tested on the 14th day of treatment (Coc.) and 48 hr following withdrawal from a 14-day administration (Coc.W). Each column is the mean of 10 determinations with s.e. mean shown by a vertical bar (calculated from original data for % time in white). V is the response of mice receiving intracerebral injection of vehicle. Significant increases or decreases in the % time spent in the white section and latency of initial movement from the white (W) to the black (B) section of the test box compared to C (nontreated cannulated mice) are indicated $*p<0.05$–$p<0.001$, reversal of the effects of Coc.W by treatment with ON are indicated $^{o}p<0.05$–$p<0.001$ (one-way ANOVA followed by Dunnett's t-test).

showed a delayed response in moving from the white to the black section and an increased time spent in the white section. Forty-eight hr following withdrawal from a 14-day treatment with cocaine, mice showed a reverse profile, moving with a reduced latency into the black section where the mice spent more time. The injection of ondansetron (10 ng) into the amygdala and dorsal raphe nucleus prevented these effects of withdrawal from cocaine, values returning to those shown by nontreated control mice (Fig. 6). Ondansetron (10 ng) injected into the medial raphe nucleus, caudate putamen or nucleus accumbens failed to prevent the behavioural changes observed after 48-hr withdrawal from cocaine (Fig. 6).

Vehicle injections into the amygdala, dorsal raphe nucleus, median raphe nucleus, the nucleus accumbens or caudate putamen did not modify the consequences of withdrawal from diazepam, ethanol, nicotine and cocaine (Figs. 2 to 6).

Histology

The brains of all cannulated animals were examined to determine the site of drug or vehicle deposition and from approximately 150 mice cannulated for each of the 5 brain areas to be investigated, only the data from every 15th animal examined are presented. An accurate placement of cannulae and subsequent injection was facilitated by the routine preparation of large

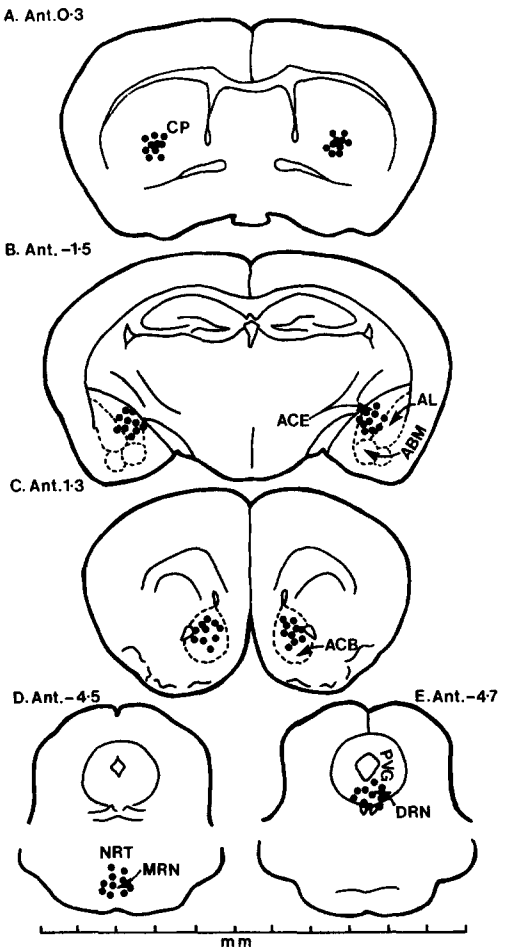

FIG. 7. Diagrammatic representation of the location of injection sites (●) in the mouse brain prepared by reference to the Atlas of Slotnick and Leonard (29), anterior coordinates indicated. Injections were directed at (A) the caudate putamen (CP), (B) the central nucleus of the amygdala (ACE), (C) the nucleus accumbens (ACB), (D) the median raphe nucleus (MRN) and (E) the dorsal raphe nucleus (DRN). Data are presented from every fifteenth brain examined; AL = nucleus amygdaloideus lateralis, ABM = nucleus amygdaloideus basalis, PVG = substantia grisea periventricularis, NRT = nucleus reticularis tegmenti pontis.

numbers of animals. The cannulae locations all were accurate to within 0.4–0.6 mm of the desired injection point and, given the small size of the structures involved, it was considered that drug or vehicle diffusion would be adequate to ensure drug action within the area of interest. Injections aimed at the central nucleus of the amygdala almost certainly influence other nuclei of the amygdala complex, e.g., the basal and lateral nuclei, and injections aimed at the medial and dorsal raphe nuclei may influence the nucleus reticularis tegmenti pontis and substantia grisea periventricularis respectively (Fig. 7).

DISCUSSION

Ondansetron administered either peripherally or by direct central injection into the amygdala or dorsal raphe nucleus is reported to reinstate behaviours suppressed by the aversive conditions of the light/dark exploration test in the mouse (11,21). This profile of action is reported for anxiolytic agents such as the benzodiazepines (14) and has been recorded during the administration of ethanol, nicotine and cocaine (10,12). An opposing profile of an exacerbation of aversive behaviour has been observed during withdrawal from treatments with diazepam, ethanol, nicotine or cocaine (10,12). The peripheral administration of ondansetron has been reported to prevent the aversive response following withdrawal from treatment with diazepam, ethanol, nicotine or cocaine in the mouse, rat and marmoset (8,9). In the present study ondansetron was found to exert a similar effect on injection into the mouse brain.

The topography of action of ondansetron followed that previously found in an assessment of its ability to reduce normal aversive behaviour in the dark/light exploration test (11). Thus, the aversive response induced by withdrawal from drugs of abuse was inhibited by the injection of ondansetron into the dorsal raphe nucleus and amygdala, but not by injections into the nucleus accumbens or striatum. Furthermore, in the previous study, injections of ondansetron into the median raphe nucleus were much less effective than injections into the dorsal raphe nucleus to reduce aversive behaviour, and this was also observed in the present study attempting to modify the aversive response caused by withdrawal from drugs of abuse. In this respect the dorsal raphe nucleus also appears particularly important for the anxiolytic activity of the benzodiazepines (16, 24, 31, 34).

Given that ondansetron is a highly potent and selective 5-HT_3 receptor antagonist, and that other 5-HT_3 receptor antagonists effectively inhibit aversive behaviour (35), the effects of ondansetron to inhibit aversive responding following withdrawal from ethanol, nicotine, cocaine and diazepam are most reasonably interpreted in terms of a 5-HT_3 receptor antagonism. Stein *et al.* (30) have hypothesised that aversive stimuli in general can activate 5-HT cells in the raphe nuclei leading to an enhanced serotonergic function in the forebrain and behavioural suppression. Certainly, there is considerable evidence that 5-HT pathways are involved in the control of aversive behaviour, with a reduction in 5-HT function leading to a release of suppressed behaviour or an 'anxiolytic' effect [see reviews by Stein *et al.* (30); Iversen (20); Gardner (17); Chopin and Briley (7)]. The amygdala, which is considered to play an important role in anxiety, receives a 5-HT innervation, and the local injection of benzodiazepines (11, 25, 27, 28) and ondansetron (11) has been shown to attenuate aversive behaviour. The present data indicate that the aversive response following withdrawal from drugs of abuse may also be mediated via the amygdala, the effect being expressed via a 5-HT_3 receptor.

Thus, in addition to an action in the midbrain, the effects of ondansetron on injection into the amygdala may indicate a forebrain site of action. The amygdala and dorsal raphe nucleus showed an equal sensitivity to the effects of ondansetron to reduce aversive responding, but it is uncertain how this is achieved in the dorsal raphe nucleus. Electrophysiological studies have shown that 5-HT inhibits the firing of 5-HT neurones in the dorsal raphe nucleus (1) and such an action would decrease 5-HT function in the forebrain. Compounds acting on the 5-HT_{1A} receptor mimic the electrophysiological effects of 5-HT in the dorsal raphe nucleus, and buspirone and related agents are thought to act as agonists or partial agonists to inhibit 5-HT cell firing to reduce aversive behaviour [see reviews by Dourish *et al.* (15); Traber and Glaser (33)]. It is clear that this particular 5-HT receptor would be inappropriate to account for the antiaversive properties of a 5-HT receptor antagonist such as ondansetron, which has no affinity for the 5-HT_{1A} receptor (6). However, the injection of 5-HT and the selective 5-HT_3 receptor agonist 2-methyl-5-HT into the dorsal raphe nucleus increases aversive responding in the dark/light exploration test in the mouse (13). The 5-HT receptor system within the dorsal raphe nucleus which mediates the effects of 2-methyl-5-HT is probably of the 5-HT_3 type and is a likely site of action for ondansetron to mediate the reduction in aversive

behaviour caused by withdrawal from drugs of abuse.

The neurochemical mechanisms by which withdrawal from drugs of abuse can precipitate an aversive response are not known. Whilst treatment with the benzodiazepines, nicotine and ethanol can modify 5-HT turnover (2, 19, 30), and cocaine can directly affect cell firing in the dorsal raphe nucleus (23), the relevance of such changes to what may occur following withdrawal from drug treatments remains to be determined.

Whilst the ability of ondansetron to release suppressed behaviour may be relevant to its effects to antagonise the behavioural consequences of withdrawal from drugs of abuse, recent experiments have shown that ondansetron can reduce alcohol consumption in the marmoset (26) and block the hyperactivity and neurochemical changes caused by activation of midbrain dopamine neurons (18). Such effects may indicate an ability to reduce craving or modify reward, and may contribute to the effectiveness of ondansetron to inhibit the behavioural consequences of withdrawal from diazepam, alcohol, nicotine and cocaine. In any event, sites of action of ondansetron are revealed in the amygdala and dorsal raphe nucleus of the mouse brain to attenuate the aversive behaviour induced by withdrawal from treatment with drugs of abuse. Such sites may represent important loci of action for ondansetron to modify behaviour following its peripheral administration.

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