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The Effects of Umespirone as a Potential Anxiolytic and Antipsychotic Agent

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BARNES, N. M., B. COSTALL, A. M. DOMENEY, P. A. GERRARD, M. E. KELLY, H. KRÄHLING, R. J. NAYLOR, D. M. TOMKINS AND T. J. WILLIAMS. *The effects of umespirone as a potential anxiolytic and antipsychotic agent.* PHARMACOL BIOCHEM BEHAV **40**(1) 89–96, 1991.—Umespirone was compared to buspirone, diazepam and clozapine as a potential anxiolytic and antipsychotic agent. In the mouse black and white test box, umespirone was considerably more potent than diazepam or buspirone to reduce aversive responding, tolerance to its effects was not observed and sedation was absent, a chronic treatment and withdrawal was not associated with an anxiogenic profile, and umespirone prevented the behavioural consequences of withdrawal from diazepam. Umespirone also had an anxiolytic profile of action in the tests of rat social interaction and in the marmoset exposed to a human threat. Both umespirone and clozapine reduced the hyperactivity induced by the infusion of dopamine into the nucleus accumbens of rat. In radioligand binding assays umespirone demonstrated nanomolar affinity for the α_1-adrenoceptor and the 5-HT_{1A} and dopamine D_2 receptors. It is concluded that umespirone may present as a novel psychotropic agent with anxiolytic and antipsychotic potential.

Umespirone Anxiolytic Antipsychotic

BY common consensus the drug treatment of anxiety and schizophrenia constitutes an important therapeutic intervention. In the majority of cases the treatment of anxiety is synonymous with benzodiazepine medication, which has important actions to facilitate gabaergic function (22,38). On the other hand, the drug therapy of schizophrenia is dominated by the neuroleptic agents, which most likely bring about their antipsychotic effects by a direct blockade of central dopamine D_2 receptors (11, 25, 27). Both groups of compounds have major clinical importance, but also have distinctive side effects: impairment of motor performance and muscle relaxation, interaction with central nervous system depressants and tolerance and withdrawal phenomena with the benzodiazepines (39) and most prominently extrapyramidal symptoms with classical neuroleptics (30). Moreover, in chronic disease, the control of negative symptoms of schizophrenia with neuroleptic agents appears to be unsatisfactory.

Against this perspective two psychotropic drugs have attracted particular interest. Buspirone has been identified as the first representative of a class of novel nonbenzodiazepine anxiolytic agents and clozapine as an atypical antipsychotic agent. Buspirone, which has no affinity for the benzodiazepine receptors, could be shown to have anxiolytic actions (20) without provoking muscle weakness, tolerance, motor impairment or withdrawal phenomena (39). The underlying mechanism of buspirone's psychotropic effects is most likely to be a modulation of central serotonergic systems (4, 10, 15, 24). Up to now two major disadvantages in use of buspirone and related compounds have been discovered, a delay in onset of action of some 1 to 2 weeks and, more importantly, a failure to substitute for benzodiazepines (39).

With clozapine, which has proven antipsychotic effects in man (32), it was shown for the first time that major dopamine D_2 receptor blocking activity is not a prerequisite for antipsychotic potential, and an impairment of extrapyramidal functioning is not an indispensable property of antipsychotic compounds (3,5). The mechanisms of clozapine's psychotropic action remains the subject of considerable discussion (32,21). The therapeutic usefulness of clozapine is, however, disadvantaged because of a certain risk of agranulocytosis.

In the present study we investigate umespirone in comparison with buspirone, diazepam and clozapine as a potential anxiolytic/antipsychotic agent, report its profile in animal models of anxiety and its ability to influence aversive behaviour following withdrawal from diazepam, and its ability to antagonise a raised mesolimbic dopamine function. In addition, we attempt to characterise the receptor binding sites of umespirone using radioligand binding assays.

METHOD

Behavioural Studies

Influence on behaviour in the mouse black:white test box. Naive BKW male albino mice (University of Bradford bred), 30–35 g, were used. Ten mice were normally housed in each cage and kept for two weeks on a 12-h light/dark cycle with the lights off at 0700 h, food and water available ad lib.

Tests for changes in behaviour were conducted 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-h period of adaptation to the new environment, they were placed individually into the centre of the white, brightly lit area of the test box.

The apparatus used for the detection of changes in exploratory behaviour consisted of an open-topped box (45 × 27 × 27 cm high) lined into 9 cm squares, two-fifths painted black and illuminated under a dim red light (1 × 60 W) and partitioned from the remainder of the box which was painted white and illuminated with a 60-W white light source located 17 cm above the box. An opening 7.5 × 7.5 cm located at floor level in the centre of the partition allowed access between the two compartments.

The mice were observed over a 5-min period by remote video-recording and four behaviours noted, 1) the number of exploratory rearings in the white and black areas, 2) the number of line crossings in the white and black areas, 3) the time spent in the white and black areas and 4) the latency of the initial movement from the white to the black area.

Mice were used once only in treatment groups of 5. Results were analysed using single-factor analysis of variance and where appropriate followed by Dunnett's procedure for comparing all treatments with control and are presented as means ± s.e.m.'s.

Long-term effects of umespirone and ability to substitute for diazepam. The effects of long-term treatment and ability to substitute for diazepam were demonstrated in the mouse model. Mice were treated twice daily with diazepam (10 mg/kg IP), buspirone (2.5 mg/kg IP) or umespirone (0.01 mg/kg PO) for 14 days. On the 14th day mice received treatment with diazepam, buspirone and umespirone (morning and night) but on the 15th day the treatment ceased. Separate groups of mice were tested during diazepam and buspirone treatment on days 3, 7 and 14, mice receiving umespirone were also tested on day 1. Separate groups of mice were tested at 8, 24, 48, 96 and 240 h after withdrawal of drug treatment.

To demonstrate the ability to substitute for diazepam a similar protocol was followed. Mice were treated twice daily with a high dose of diazepam (10 mg/kg IP) for 7 days. On the 8th day the diazepam treatment was replaced by vehicle, umespirone or buspirone. Treatments with vehicle (1 ml/100 g saline, IP) umespirone (0.01 mg/kg PO) or buspirone (1 mg/kg IP) were given twice daily during the period of diazepam withdrawal. Separate groups of mice were tested on day 7 during diazepam treatment and 24 h after withdrawal.

Doses for these studies were selected from the dose-response analyses to acute administrations. Mice were used once only in treatment groups of 5. Results were analysed using single-factor analysis of variance and where appropriate followed by Dunnett's procedure for comparing all treatments to control and are presented as mean ± S.E.M.s.

Influence on rat social interaction. Male Sprague-Dawley rats (University of Bradford bred), 225–275 g, were normally housed in groups of 5 and kept on a 12-h light/dark cycle with lights on at 0800 h. Tests were conducted between 1300 and 1800 h in an illuminated room.

The apparatus used for the detection of changes in rat social interaction and exploratory behaviour consisted of a Perspex open-topped box (51 × 51 cm and 20 cm high) with 17 × 17 cm areas marked on the floor. Two naive rats, from separate housing cages, were placed into the box (with a 60-W bright white illumination 17 cm above) and their behaviour observed over a 10-min period by remote video recording. Two behaviours were noted, 1) social interaction between the animals was determined

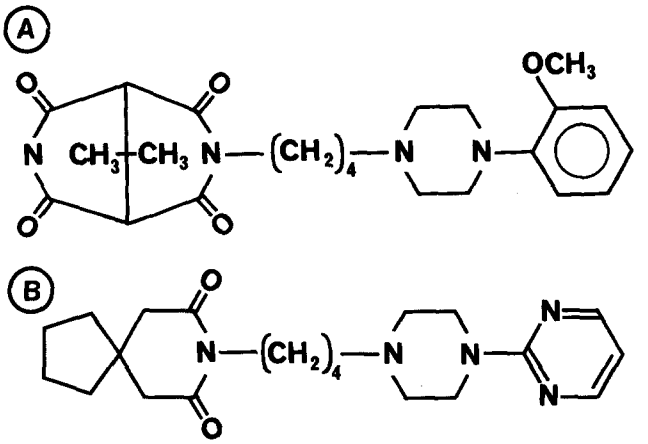

FIG. 1. Structures of (A) umespirone and (B) buspirone.

by timing (s) sniffing of partner, crawling under or climbing over partner, genital investigation of partner, following partner and 2) exploratory locomotion was measured as the number of crossings of the lines marked on the floor of the test box. Values for time spent in social interaction and moving around the observation cage were determined for individual animals. Naive animals were used in drug treatment pairs of 5–6. Data obtained were analysed using single-factor analysis of variance followed by Dunnett's *t*-test and are presented as means ± s.e.m.s.

Influence on behaviour of the marmoset exposed to a human threat situation. Male and female laboratory bred common marmoset (*Callithrix jacchus*), 350–440 g, were housed in single sex pairs. Holding rooms were maintained at 25 ± 1°C at a humidity of 55% and on a 12-h light/dark cycle (with simulated dawn and twilight periods, red illumination) with lights on at 0700 h. Tests were conducted between 1330 and 1530 h in the normal holding room (to avoid unwanted disruption of behaviour by movement to a novel cage).

The holding cages measured 75 cm high, 50 cm wide and 60 cm deep. A behavioural change characterised by retreat from, and posturing towards a human threat (a behaviour sensitive to known anxiolytic agents) was initiated by a human observer standing in close proximity (60 cm) to the holding cage. Changed behaviour was recorded over a 2-min period by the observer using a keypad connected to a BBC microcomputer. The behavioural measures selected for the present study were, 1) the % of time the animals spent forward of the front perch in direct confrontation with the human threat and 2) the number of body postures, primarily shown as raising of the tail to expose the genital region, anal scent marking and slit stare with flattened ear tufts.

Twelve marmosets were used at 7-day intervals throughout the study and were subject to a random crossover of treatments. Buspirone (0.05–1.0 mg/kg), diazepam (1–25 μg/kg), umespirone (1–100 μg/kg) or vehicle (saline 1 ml/kg) were given SC 40 min before testing.

Data were analysed using paired *t*-test and are presented as mean number of postures ± s.e.m.s and % time forward.

Influence on a mesolimbic dopamine hyperactivity response. The studies used female Sprague-Dawley (University of Bradford bred) rats weighing 275 ± 325 g at the time of initial surgery. Rats housed in groups of 5 and kept on a 12-h light/dark cycle with lights on at 0800 h, food and water available ad lib. Standard stereotaxic techniques were used for the implantation

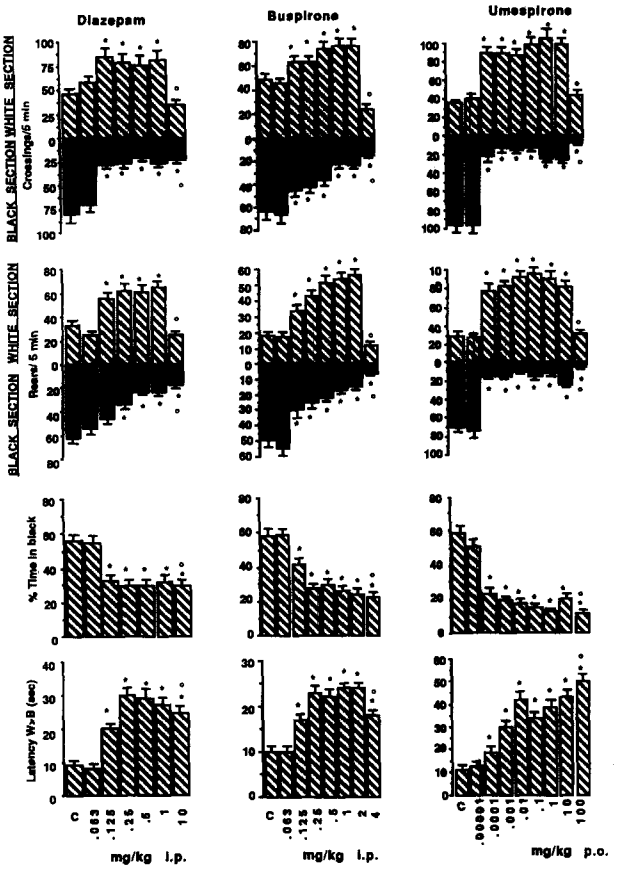

FIG. 2. The effect of diazepam, buspirone and umespirone given acutely in the black/white exploration test in the mouse. Testing was carried out 45 min after dosing and mouse rearing behaviour, line crossings, the % time spent in the white (W) and black (B) sections and the latency of initial movement from the white to the black section were recorded over a 5-min period. C indicates the response of vehicle-treated controls. Values represent the mean ± s.e.m.s of 5 determinations, s.e.m.s for % time spent in the black compartment were calculated from original data. Significant increases or decreases in responding compared to control values are indicated *$p<0.05$, $p<0.05$–0.01 (one-way ANOVA followed by Dunnett's t-test). °Sedation.

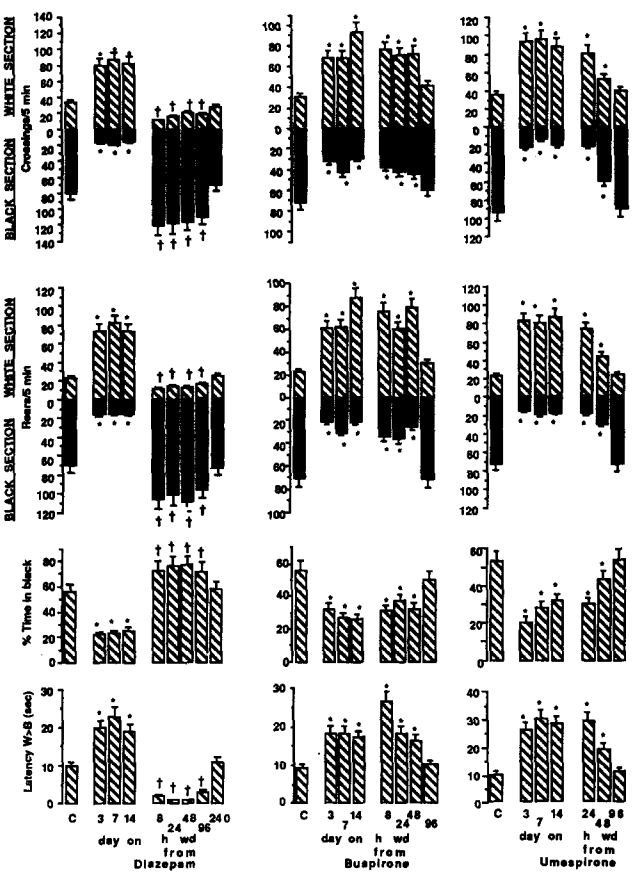

FIG. 3. The effects of diazepam, buspirone and umespirone, given chronically or withdrawn from chronic treatment in the black/white exploration test in mouse. Testing was carried out on the 3rd, 7th or 14th day of treatment (chronic, diazepam 10 mg/kg IP b.i.d.; buspirone 2.5 mg/kg IP b.i.d.; umespirone 0.01 mg/kg PO b.i.d.) and up to 240 h of withdrawal (wd) from chronic treatment. n = 5 per group. s.e.m.s of the means are given and were calculated from original data for % time spent in the black compartment. *$p<0.01$ for redistribution of behaviour in favour of the light section, †$p<0.01$ for redistribution in favour of the dark compared to control (C) values (one-way ANOVA followed by Dunnett's t-test).

of chronically indwelling guide cannulae for subsequent bilateral intracerebral infusion into the centre of the nucleus accumbens [anterior 9.4, vertical 0.0, lateral ± 1.6, atlas of De Groot (13)]. Rats were anaesthetised with chloral hydrate (200 mg/kg SC) and placed in a Kopf stereotaxic instrument. Implanted guides were constructed of stainless steel, 0.65 mm diameter, held bilaterally in Perspex holders. Guides terminated 3.5 mm above the centre of the nucleus accumbens and were kept patent for a 14-day recovery period using stainless steel stylets extending 0.5 mm beyond the guide tips.

After 14 days recovery, rats were anaesthetised with halothane/N_2O, O_2 for the SC implantation in the scapula region of two Alzet osmotic minipumps each attached via 3–4 mm PP25 and 40–45 mm PP60 polythene tubing to stainless steel injection units (0.3 mm diameter) which were made to fit permanently into the previously implanted guides in place of the stylets, but terminating 3.5 mm below the guide tips at the centre of the nucleus accumbens. The pumps had previously been filled with dopamine solution (2.17 μg/μl dopamine hydrochloride prepared in N_2 bubbled solution containing 0.1% sodium metabisulphite), or its solvent, and the entire injection unit primed overnight at 37°C. The pumps delivered dopamine or its solvent at a constant rate of 0.48 μl/h from the time of implantation, and thus provided an intra-accumbens dose of dopamine of 25 μg over a 24-h period. Pumps were removed on day 13.

Rat spontaneous locomotion was measured using individual photocell cages, banked in groups of 30. Each cage was constructed of Perspex, 25 × 15 × 15 cm high, fitted with one photocell unit placed off-centre. The cages were screened. Interruptions of the light beams were recorded every 5 min and cumulated to give a value for locomotion activity in counts/60 min. Rat spontaneous locomotion was generally measured between 0800 and 1100 h.

Biochemistry

Rat brain tissue was obtained from female Hooded-Lister rats (250–300 g); individual nuclei were dissected and homogenised

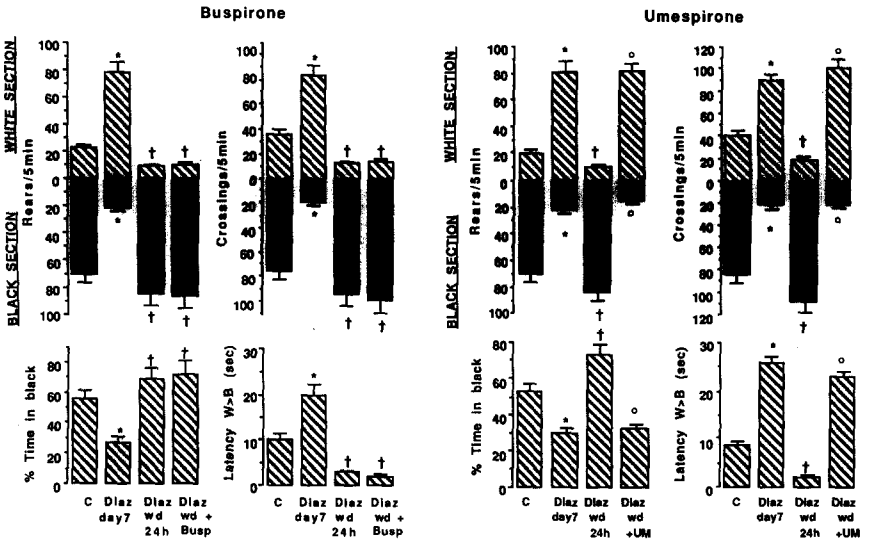

FIG. 4. The effects on mouse black/white exploration of chronic (7-day) treatment with diazepam (10 mg/kg IP b.i.d.), effects of withdrawing from diazepam (diaz WD) and the influence of buspirone (1 mg/kg IP b.i.d.) and umespirone (0.01 mg/kg PO b.i.d.) on the behavioural consequences of withdrawal from diazepam (diaz WD + busp, diaz WD + UM). n = 5, s.e.m.s are given; for % time spent in the light these were calculated from original data. *$p<0.01$ for redistribution of behaviour in favour of the light section, †$p<0.01$ for redistribution in favour of the dark, °$p<0.01$ for inhibition of the behavioural consequences of withdrawing from diazepam treatment (one-way ANOVA followed by Dunnett's *t*-test).

in 20 volumes wt./vol. ice-cold 50 mmol/l Hepes buffer, pH 7.4. The homogenate was centrifuged at 48,000 × g for 10 minutes at 4°C and the pellet was washed by resuspension and recentrifugation. The binding homogenate was formed by resuspending the pellet in Hepes buffer at a concentration of 80–120 mg original wet weight/ml.

For competition studies 650 µl displacing drug or buffer was added to the assay tubes followed by 100 µl [^{3}H]umespirone [51 Ci/mmol (Amersham), final concentration 0.9–1.1 nM] in Hepes buffer. Two hundred and fifty µl of brain tissue homogenate was added to initiate binding, which was allowed to proceed for 30 minutes at 37°C. The incubation was terminated by rapid filtration through prewet Whatman GF/B filters followed by washing with 7.5 ml buffer. Bound radioactivity was assessed by liquid scintillation spectroscopy. Each individual assay was performed in triplicate and the incubation stage was completed within 60 minutes of preparing the membranes.

For competition studies using [^{3}H]8-OHDPAT (Amersham) (0.5 nM) and [^{3}H]haloperidol (N.E.N.) (1.0 nM) the membrane homogenates (rat cortex and cerebellum respectively) were prepared as above. The buffer used in both studies was 50 mmol/ Hepes (pH 7.4). In the [^{3}H]haloperidol studies the buffer also contained 120 mM NaCl, and spiperone (25 nmol/l) was included to mask dopamine D_2 receptors.

Drugs

Buspirone hydrochloride (Bristol-Myers) was prepared in saline, clozapine (Sandoz) was prepared with the minimum of hydrochloric acid and made up to volume with distilled water. Diazepam (Roche) was prepared in the minimum of PEG and made up to volume with saline. For umespirone [KC9172, (3-butyl-9,9-dimethyl-7-[4-[4′(2-methoxyphenyl-1-piperazinyl]butyl]-3,7-diazabicyclo [3.3.1]nonan-2,4,6,8-tetraone; Fig. 1; batch Al/M; Kalichemie AG, Hannover, FRG] a suspension/solution was prepared as follows: 1 l of solution to suspend umespirone was

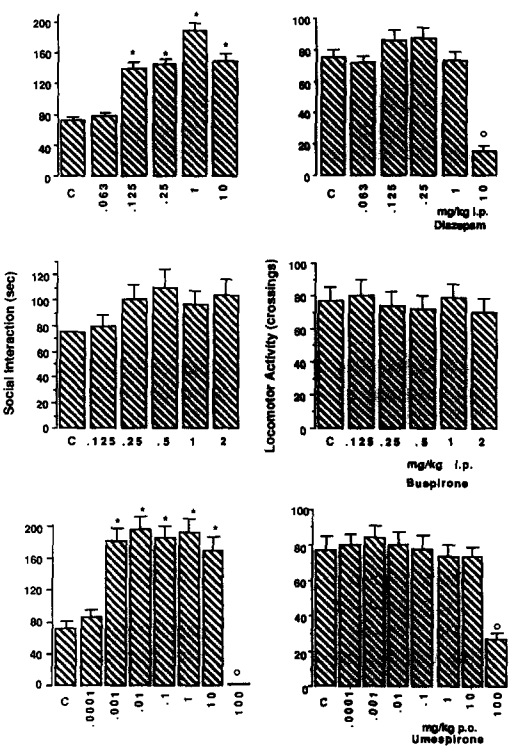

FIG. 5. The effects of diazepam, umespirone and buspirone on social interaction and locomotion activity (line crossings) in rats. Testing was carried out for a 10-min period 45 min after drug treatment. n = 5–6 pairs per group. Standard errors of the means are given. *$p<0.01$ compared to controls (one-way ANOVA followed by Dunnett's *t*-test). °Sedation.

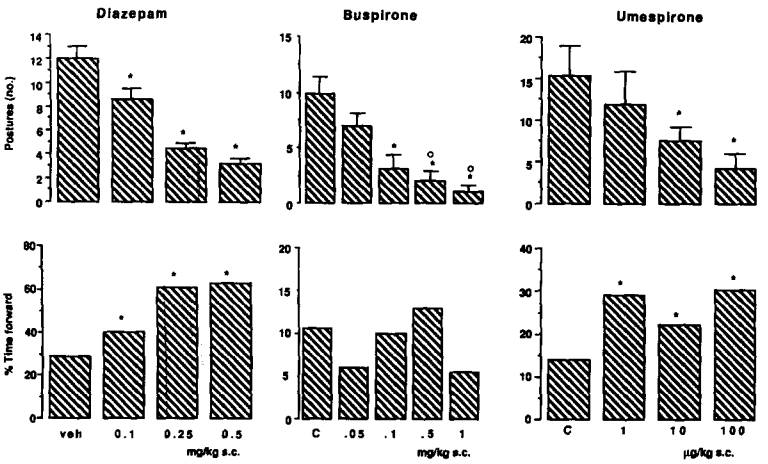

FIG. 6. The effects of diazepam, buspirone and umespirone on anxiety-related behaviours in marmosets. Treatments were administered SC 45 min before the behavioural observations were made. The marmosets were confronted by an observer standing in close proximity to the home cage and measurements of the number of postures made and time spent forward of the front perch recorded over a 2-min period. n = 4 per group. Vertical bars indicate s.e. of means. *$p<0.05$–$p<0.001$ compared to vehicle-treated controls (C) (paired t-test). °Sedation.

prepared by adding 20 g Tylose MH50 to 300 ml distilled water at 70°C and with stirring 2 g Tween 80 was added with distilled water to give 1000 ml; the solution was left for 2 h in a refrigerator. The suspension of umespirone was prepared by adding an appropriate amount of umespirone to a small volume of the above medium and mixing thoroughly with the aid of ultrasonics; the solution was diluted to useable doses with saline.

Drugs were administered in a volume of 1 ml/kg (rats, marmosets) or 1 ml/100 g (mice). All doses are expressed as the base.

RESULTS

Influence on Behaviour in the Mouse Black:White Test Box

Effects of acute drug administrations. The action of diazepam (0.125–1.0 mg/kg IP), buspirone (0.125–2.0 mg/kg IP) and umespirone (0.1 µg–10 mg/kg PO) was shown as a reduced aversion for the white, brightly lit area. This was demonstrated as a decrease in the percentage of time spent in the black section, a delay in the latency of initial movement from the white to the black section and increased rearings and line crossings in the white area (Fig. 2). The degree of behavioural change was similar for all three agents. At higher doses, diazepam (10 mg/kg IP) buspirone (4 mg/kg IP) and umespirone (100 mg/kg PO) induced sedation that reduced behaviour in both the black and white areas, obscuring an interpretation of changes in aversive behaviour (Fig. 2).

Effects of long-term drug treatments and withdrawal. Longer term treatment with diazepam (10 mg/kg IP b.i.d.), buspirone (2.5 mg/kg IP b.i.d.) and umespirone (0.01 mg/kg PO b.i.d.) for 14 days also caused a redistribution of behaviour to the white area of the test box. However, within 8 h of withdrawal of diazepam treatment, the profile of responding was a decrease in the time spent in the white area, a decrease in latency to move from the white to the black section and a decrease in line crossings and rearings in the white area. This redistribution of behaviour to the black area of the test box was not observed following withdrawal from treatment with either buspirone or umespirone, the enhanced responding in the white area persisting for 48 h (buspirone) or decreasing to control values over 96 h (umespirone) (Fig. 3).

Ability of umespirone to substitute for diazepam. Umespirone (0.01 mg/kg PO b.i.d.) administered during the period of withdrawal from diazepam (10 mg/kg IP b.i.d. for 7 days) prevented the redistribution of behaviour to the black area of the test box (Fig. 4). In contrast, buspirone (1 mg/kg IP b.i.d.) administered during the period of withdrawal from diazepam failed to prevent the exacerbation in aversive responding to the white area of the test box (Fig. 4).

Influence on rat social interaction. The amount of time spent in active social interaction under conditions of high light unfamiliarity was increased by diazepam (0.125–1.0 mg/kg IP) at doses which failed to influence locomotor activity (line crossings); sedation developed at 10 mg/kg IP to interfere with the measurement of social interaction (Fig. 5). Umespirone was also shown to increase rat social interaction over an extended dose range (0.001–10 mg/kg PO) that failed to suppress locomotor activity. A higher dose of 100 mg/kg caused sedation to nonspecifically modify behaviour (Fig. 5). In contrast, buspirone (0.1–2 mg/kg IP) failed to significantly elevate social interaction using the present test conditions (Fig. 5), and doses of 4 and 8 mg/kg IP caused sedation.

Influence on behaviour of the marmoset exposed to a human threat. Following treatment with diazepam (0.1–0.5 mg/kg SC), the number of postures exhibited by the marmosets in response to a human threat were reduced and the % of time spent forward of the front perch in confrontation with the experimenter increased (Fig. 6). Umespirone (10 and 100 µg/kg SC) induced changes of a similar magnitude: postures were significantly reduced and the % time forward of the front perch significantly increased. At a lower dose of 1 µg/kg SC, 4 marmosets gave a response similar to that of higher doses, whilst this was insignificant or absent in the remaining 4 animals (Fig. 6). A different profile was observed using buspirone; a reduction was observed in the number of postures but there was no significant change in the % of time forward of the front perch (Fig. 6). The reduc-

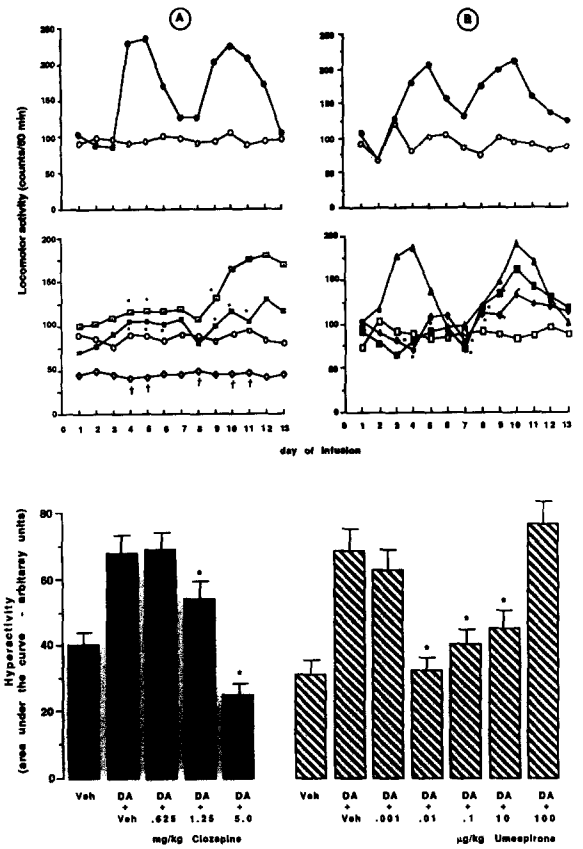

FIG. 7. Antagonism by (A) clozapine and (B) umespirone of the locomotor hyperactivity caused by the bilateral infusion of dopamine into the nucleus accumbens of the rat. The locomotor activity of animals was measured during the 13-day period of dopamine (25 µg/24 h, ● - ●) or vehicle (○ - ○) infusion, or dopamine plus clozapine (□ - □, 0.6; ■ - ■ 1.25; ◇ - ◇ 5.0 mg/kg IP b.i.d.) or vehicle (○ - ○) or dopamine plus umespirone (▲ - ▲, 1.0 ng; ◆ - ◆, 10 ng; □ - □, 100 ng; ■ - ■ 10 µg/kg IP b.i.d.). Each value is the mean of 5 determinations; s.e.m.s were in the range 9.7 to 22% and to improve clarity of presentation are omitted. Significant reductions in activity compared to the dopamine control values (upper diagrams) are indicated *$p<0.05$; a significant reduction below vehicle control values is indicated †$p<0.05$ (two-way ANOVA followed by Dunnett's t-test for multiple comparisons). The histograms indicate the dose-response relationships for clozapine and umespirone to antagonise the dopamine-induced hyperactivity response. The locomotor activity is presented in arbitrary units as areas under the hyperactivity curves for 13 days. Each value is the mean obtained from 5 animals. A significant antagonism of the dopamine-induced hyperactivity is indicated *$p<0.05$ (two-way ANOVA followed by Dunnett's t-test for multiple comparisons).

tion in postures was significant within the dose range 0.1–1.0 mg/kg SC but sedation was apparent at the doses of 0.5 and 1.0 mg/kg SC.

Influence on a mesolimbic dopamine hyperactivity response. The persistent infusion of dopamine (25 µg/24 h) into the rat nucleus accumbens lead to a biphasic hyperactivity response, with peaks generally occurring between days 3 to 5 and 9 to 11 (Fig. 7). As a prototype atypical antipsychotic agent, clozapine, at a low dose of 0.6 mg/kg IP b.i.d. abolished the first peak of hyperactivity with a nonsignificant reduction in the magnitude of the second peak. A higher dose of 1.25 mg/kg IP b.i.d. clozapine suppressed both peaks of activity to values not significantly different from vehicle-treated controls. As the dose was increased to 5 mg/kg IP b.i.d. then locomotor responding was reduced to values significantly below those of vehicle treated controls (Fig. 7).

Umespirone was shown to be at least 10,000 times more potent than clozapine to inhibit the dopamine-induced hyperactivity response. At the low dose of 1 ng/kg IP b.i.d. umespirone control of the dopamine response was complete in 3/5 animals and there was some breakthrough response in 2 animals resulting in a confirmed mean response. At 10 ng the antagonism was complete in all animals, but at higher doses of 0.1, 10 and 100 µ/kg IP b.i.d. the blockade of the dopamine response became less and was lost completely in all animals treated with 100 µg/kg IP b.i.d., causing a U-shaped dose-response curve (Fig. 7).

Characterisation of umespirone binding sites using radioligand binding assays. Compounds with a range of pharmacological actions competed for the recognition sites labelled by 1.0 nM [^{3}H]umespirone in rat brain homogenates. The level of specific binding at this concentration of umespirone (as defined by 1 µM unlabelled umespirone) was approximately 85–90% of total binding. Compounds with high affinity for α_1 adrenoceptors (prazosin, benoxathian, phentolamine) produced biphasic inhibition curves, the high affinity component apparently competing for an α_1 adrenoceptor recognition site labelled by [^{3}H]umespirone (mean pIC_{50} values were 9.30, 8.70 and 8.15 respectively). The α_1 site comprised approximately 40–45% of total 1.0 nM [^{3}H]-umespirone binding in rat cortical homogenates. In subsequent studies prazosin (30 nM) was included in the buffer to mask this site. In the presence of prazosin the dopamine D_2 receptor antagonists haloperidol and sulpiride competed with high affinity for approximately 40–50% of 1 nM [^{3}H]umesprione binding to striatal homogenates, haloperidol being approximately 20-fold more potent than sulpiride in this respect with mean pIC_{50} values of 8.69 and 7.39 respectively.

Also, in the presence of prazosin, a compound with high affinity for 5-HT_{1A} recognition sites, namely 8-OHDPAT, potently competed for approximately 20% of 1 nM umespirone binding to cortical homogenates, with a mean pIC_{50} value of 8.70. In competition experiments using [^{3}H]8-OHDPAT (0.5 nM), unlabelled umespirone competed with this radioligand for binding sites in rat brain cortical homogenates, with a mean pIC_{50} value of 7.64 (yielding a K_i value of 15.7 nM). The following compounds did not demonstrate any high-affinity (<100 nM) component of competition for 1.0 nM [^{3}H]umespirone binding to rat brain membranes; idazoxan, propranolol, SCH23390, ritanserin, ondansetron, (+)3PPP, pentazocine, chlordiazepoxide, mepyramine, ranitidine, hexamethonium, atropine. In addition, unlabelled umespirone, at concentrations up to 10 µM, did not compete for sigma recognition sites labelled by 1.0 nM [^{3}H]haloperidol.

DISCUSSION

In three test procedures which are sensitive to the actions of known anxiolytic agents, mouse aversion to a brightly lit environment (9, 10, 12), social interaction in the rat (16) and responsiveness to a human threat in the common marmoset (28), umespirone demonstrated an anxiolytic profile of action. Thus, in the rodent, both umespirone and diazepam reduced mouse aversion to the brightly illuminated compartment of the test box and increased social interaction in unfamiliar rats; umespirone was 100 to 1000 times more potent than diazepam. In the marmoset it had a comparable potency to diazepam to reduce posturing towards a human threat or retreat from a human threat. Umespirone was also more potent than buspirone in the mouse

and marmoset tests and in the latter model buspirone failed to influence the time spent forward of the front perch. Umespirone was also distinguished from buspirone by the latter's failure to influence rat social interaction and the inconsistent actions of buspirone in animal models of anxiety have been noted elsewhere (10, 18, 34).

The consistent profile of action of umespirone as an anxiolytic agent in three species prompted further analysis of its effects on repeated treatment and withdrawal, and its ability to substitute with diazepam. Using the mouse black and white test box, in the present and previous studies (2), withdrawal from treatment with diazepam caused an increased aversion to the white area of the test box: this is indicative of an anxiogenic response (2). In contrast, withdrawal from either buspirone or umespirone was followed by a gradual return of values to control levels shown by nondrug-treated mice; an anxiogenic profile was absent. However, a clear difference was observed between buspirone and umespirone in an attempt to prevent the behavioural consequences of withdrawal from diazepam since only umespirone could prevent the anxiogenic response. Tolerance to the effects of umespirone on repeated treatment was not observed. Therefore, umespirone is indicated to have a highly potent action in animal models of anxiety, which lacks tolerance, that does not precipitate an aversive behaviour on withdrawal from chronic treatment, yet can prevent the behavioural consequences of withdrawal from diazepam.

In addition to its actions in anxiety models, umespirone was also shown to be a potent agent to reduce the hyperactivity induced by the infusion of dopamine into the nucleus accumbens of the rat. Classical neuroleptic agents such as haloperidol (7), but also atypical antipsychotic agents such as sulpiride (7) and clozapine (present study) and 5-HT_3 receptor antagonists (8), are effective antagonists of the dopamine response. However, unlike clozapine and neuroleptic drugs, umespirone at higher doses did not antagonise the dopamine-induced hyperactivity response to below control values. Indeed, at higher doses the antagonism decreased, causing a U-shaped dose response curve. However, similarly to clozapine, umespirone fails to induce catalepsy or antagonise drug-induced stereotypy (29,36) and mimics the action of clozapine to inhibit the sniffing behaviour induced by the NMDA antagonist AP-5 (37).

Thus umespirone is indicated to have an anxiolytic and antipsychotic profile of action, and radioligand binding was used in an attempt to assess its possible mechanism(s) of action. From the results of the present studies, umespirone appears to label with similar high (nanomolar) affinity a combination of dopamine D_2 receptor, 5-HT_{1A} receptor and α_1-adrenoceptor recognition sites in homogenates of rat brain. This is in agreement with previous studies on this compound, which reported affinities (K_i values) of umespirone at these three sites of 23 nM, 15 nM and 14 nM respectively (Krähling et al., personal communication). The affinity of umespirone for dopamine D_2 receptors may account in part for its ability to antagonise limbic dopamine function, though the present studies have demonstrated marked differences between the behavioural profile of this compound and those produced by other neuroleptic agents. The affinity of umespirone for the 5-HT_{1A} recognition site may contribute importantly to the anxiolytic actions of the piperazinyl derivative umespirone and be related to the actions of other agents from the series such as buspirone. The demonstration that buspirone and other analogues, e.g., ipsapirone and gepirone, have high affinity for the 5-HT_{1A} receptor (19, 23, 33, 35) has focused interest on the relevance of the serotonergic system as a site of drug action in anxiety [see (15); and reviews (6, 17, 18, 23, 40)]. There is considerable evidence from behavioural, biochemical and electrophysiological studies that buspirone and its analogues may induce their effects via a 5-HT involvement mediated through an agonist/partial agonist action at the 5-HT_{1A} receptor (1, 4, 10, 14, 24, 30, 41, 42).

The affinity of umespirone for specific sites is emphasised by the failure of other neurotransmitter receptor ligands (affecting β- and α_2-adrenoceptors, 5-HT_2, DA_1, opiate, benzodiazepine, histamine and acetylcholine receptors) to influence umespirone binding. Whilst the behavioural spectrum of umespirone mimics the actions of the 5-HT_3 receptor antagonists (9,28), ondansetron failed to compete with [^{3}H]umespirone except at micromolar concentrations. The significance of the binding of umespirone and buspirone to the sigma receptor (26) remains to be determined since the present studies failed to show any affinity of umespirone at this recognition site.

Thus, in summary, umespirone is revealed to have affinity for a number of transmitter receptor sites and some remain to be fully characterised. An affinity for the 5-HT_{1A} site may be important for the anxiolytic actions, whereas its actions to reduce dopamine hyperactivity remain to be determined in more depth. Schmidt et al. (37) have suggested that the latter actions may involve a modulation of the cortico-subcortical glutamatergic pathways. In any event, the actions of and differences between buspirone, umespirone and related piperazinyl derivative, make such agents important tools in an understanding of the mechanisms controlling 5-HT and dopamine systems, and the development of novel anxiolytic and perhaps antipsychotic agents.

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