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Zacopride: anxiolytic profile in rodent and primate models of anxiety

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Abstract—Zacopride, a substituted benzamide derivative, was compared with diazepam in three models of experimental or provoked anxiety. The drug's action (i) in reducing aversion to a brightly lit environment was assessed in mice using a two compartment black and white test box system, (ii) in disinhibiting a suppressed behaviour was measured in the rat social interaction test under high light/unfamiliar conditions and (iii) in antagonizing a defensive response in the marmoset was assessed using the threat of a human presence. Both zacopride and diazepam enhanced exploratory behaviour and social interaction in the mouse and rat models and antagonized the defensive response in the marmoset, zacopride being 100 times more potent than diazepam. It is concluded that the 5-HT_3 receptor antagonist, zacopride, alters rodent and primate behaviour in a manner consistent with that of an anxiolytic agent.

The development of selective 5-HT_3 receptor antagonists based on a tropane (e.g. MDL 72222), indole (e.g. ICS 205-930) or carbazolone (GR38032F) nucleus has played a crucial role in the characterization of 5-hydroxytryptamine (5-HT) receptors in peripheral systems (see review by Fozard 1984; Richardson et al 1985; Bradley et al 1986; and Brittain et al 1987). In addition, such compounds are being used to assess the functional significance of 5-HT_3 receptors, which appear to be involved with gastric emptying and emesis (Buchheit et al 1985; Miner & Sanger 1986; Costall et al 1986, 1987a). Furthermore, 5-HT_3 receptor antagonists from the above series have profiles of anxiolytic action in rodent and primate models (Costall et al 1987b; Jones et al 1987; Tyers et al 1987) and high affinity binding sites have recently been found for the 5-HT_3 receptor antagonist GR65630 (3-(5-methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone) in the rat brain (Kilpatrick et al 1987). The data indicates that 5-HT_3 receptor antagonists may have potential to influence both peripheral and central 5-HT function.

Zacopride, a substituted benzamide derivative (USA patent 4657911 assigned to Delalande S.A.), has been shown to enhance gastric emptying and to antagonize the emesis induced by cytotoxic therapy (Alphin et al 1986; Smith et al 1986). Subsequent studies have shown that zacopride can antagonize the actions of 5-HT on the vagus nerve, von Bezold Jarisch reflex and ileum (Smith et al 1988) and in both the in-vivo and in-vitro tests the potency of zacopride as a 5-HT_3 receptor antagonist is comparable with that of ICS 205-930 and GR38032F. In the present study we use zacopride to investigate whether the anxiolytic action of the 5-HT_3 receptor antagonists can be extended to compounds from the substituted benzamide series.

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We report that zacopride alters behaviour in mouse, rat and marmoset models in a manner consistent with a potent and effective anxiolytic agent.

Materials and methods

Influence on behaviour in the mouse black: white test box

Naive B.K.W. male albino mice, 30–35 g, were used. 10 mice were normally housed in each cage and kept for two weeks on a 12 h light/dark cycle with lights off at 0700 h. Tests for changes in behaviour which are known to be influenced by anxiolytic agents were conducted between 1300 and 1800 h 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 × 60W) and partitioned from the remainder of the box which was painted white and brightly illuminated with a 60W 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, (i) the number of exploratory rearings in the white and black sections, (ii) the number of line crossings in the white and black areas, (iii) the time spent in the white and black areas and (iv) the latency of the initial movement from the white to the black area.

Mice were used once only in treatment groups of five. Results were analysed using single-factor analysis of variance and where appropriate followed by Dunnett's procedure for comparing all treatments with control.

Influence on rat social interaction

Male Sprague-Dawley rats, 225–275 g, were normally housed in groups of five and kept on a 12 h light/dark cycle with lights on at 0800 h. Tests were conducted between 1300–1800 h in an illuminated room. The apparatus used for the detection of changes in rat social interaction and exploratory behaviour consisted of an opaque white Perspex open-topped box (45 × 32 cm and 20 cm high) with 15 × 16 cm areas marked on the floor. Two naive rats, from separate housing cages, were placed into the box (with a 100W bright white illumination 17 cm above) and their behaviour observed over a 10 min period by remote video

recording. Two behaviours were noted, (i) social interaction between the animals was determined by timing (seconds), sniffing of partner, crawling under or climbing over partner, genital investigation of partner, following partner and (ii) 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 treated pairs in treatment groups of six, i.e. twelve animals. Data obtained were analysed using single-factor analysis of variance followed by Dunnett's *t*-test.

Influence on behaviour of the marmoset exposed to a human threat situation

Male and female laboratory bred common marmosets (*Callithrix jacchus*), 350–400 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 twilight periods, red illumination) with lights on at 0700 h. Tests were conducted between 1330–1530 h in the normal holding room (to avoid unwanted disruption of behaviour by movement to a novel room or cage). The holding cages measured 75 cm high, 50 cm wide and 60 cm deep. A behavioural change characterized 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 in front of the holding cage. Changed behaviour was recorded over a 2 min period by the observer. The behavioural measures selected for the present study were, (i) the % of time spent on the cage front in direct confrontation with the human threat and (ii) the number of body postures, primarily shown as raising of the tail to expose the genital region with varying degrees of body piloerection, anal scent marking and slit stare with flattened ear tufts (see Stevenson & Poole 1976).

12 marmosets were used at 7 day intervals throughout the study and were subject to a random cross-over of treatments. Statistical analysis utilized a one-way analysis of variance followed by Dunnett's *t*-test. Pretreatment times for zacopride and diazepam were 40–45 min.

Zacopride (4-amino-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-5-chloro-2-methoxybenzamide(E)-2-butenedioate (A. H. Robins Company Ltd.) was prepared in sterile saline (0·9% NaCl), and diazepam (Roche) was dissolved in the minimum quantity of polyethylene glycol and prepared to volume with sterile saline. Doses, expressed as the base, were administered in a volume of 1 mL kg^{-1} i.p. (rat), 1 mL kg^{-1} s.c. (marmoset) and 1 mL/100 g i.p. (mouse).

Results

Influence on behaviour in the mouse black: white test box

Under the test conditions described, control vehicle-treated mice showed a characteristic profile of (i) a latency of 12 ± 1·1 s to move from the white to the black area of the test box on first being placed into the brightly lit section, (ii) a consistent 52–54% of test time in the black section of the test box, (iii) more behavioural exploration in the black section was measured as exploratory rears (38·4 ± 2·9/5 min in the black compared with 13·2 ± 2·8/5 min in the white) or (iv) as line crossings (71·7 ± 6·5/5 min in the black compared with 49·3 ± 5·2/5 min in the white (see Fig. 1). This distribution of behaviour may reflect the aversive properties of the brightly lit area. Comparable control data are presented in Fig. 2.

Both zacopride and diazepam selectively enhanced exploratory activity in the white area of the test box. Thus, on placing the mice into the white area there were (i) significant delays in the latency to move into the black area and, using maximally effective doses of zacopride or diazepam, (ii) a 40% increase in the time spent in the white section, (iii) an approximate 4-fold increase in rearing behaviour in the white section and corresponding 3- to 4-fold reductions in the black area and (iv) approximate 1·5- to 2-fold increases in line crossings in the white area with corresponding reductions in the black section (Figs 1, 2). It is clear that the increase in exploratory behaviour (rearing) in the white area was considerably in excess of that which could have been expected from the increased time spent in the brightly lit section, whereas a significant component of the increase in line crossings may be attributed to the increased time spent in the white area. Whilst the profiles of action of zacopride and diazepam were similar, zacopride was at least one hundred times more potent than diazepam and there was no evidence of any sedative effects over a wide dose range (0·0001–10 mg kg^{-1} i.p.). In contrast, the sedative effects of diazepam at 10·0 mg kg^{-1} obscured the changes in anxiety responding. This profile of

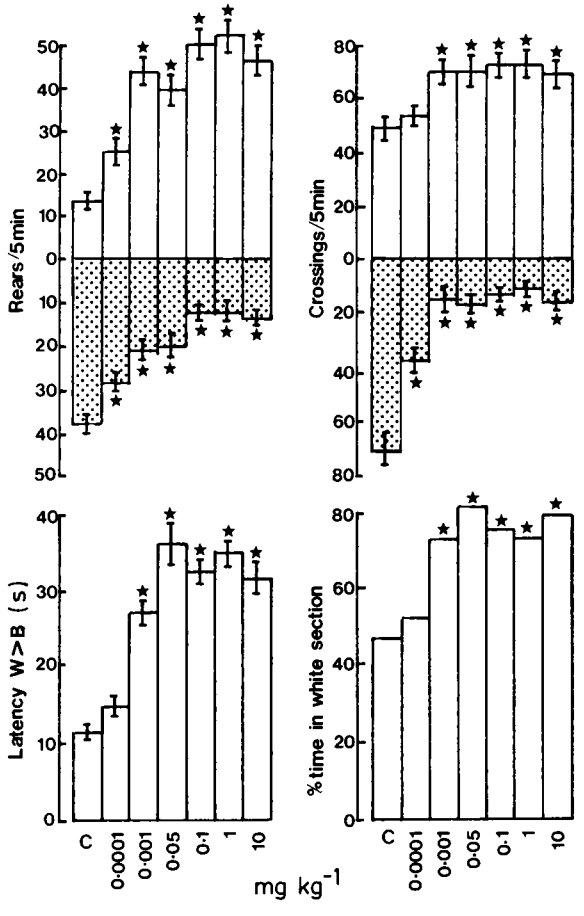

FIG. 1. Disinhibitory effects of zacopride in the light/dark discrimination test in the mouse. Mice were tested singly in an open topped box, three-fifths painted white and brightly illuminated (white section) and partitioned (with an interconnecting door) from the remainder of the box which was painted black and illuminated with red light (dark section). The floor of each section was lined into 9 cm squares. Behavioural changes in rearing (rears), line crossings (crossings), latency of movement from the white (W) to the black (B) section (after first placement into the white area) and % time spent in the white area were recorded. In the upper set of histograms, hatched columns indicate data for the dark area, open columns for the light area. Data obtained from control (C) and drug treated mice were analysed using single factor analysis of variance, and Dunnett's *t*-test. Significant increases/decreases in responding are indicated as $*P<0·05$–$P<0·001$. n = 5. Vertical bars indicate s.e.m.s; s.e.m.s were less than 12% on the original data for calculation of the % time in white section.

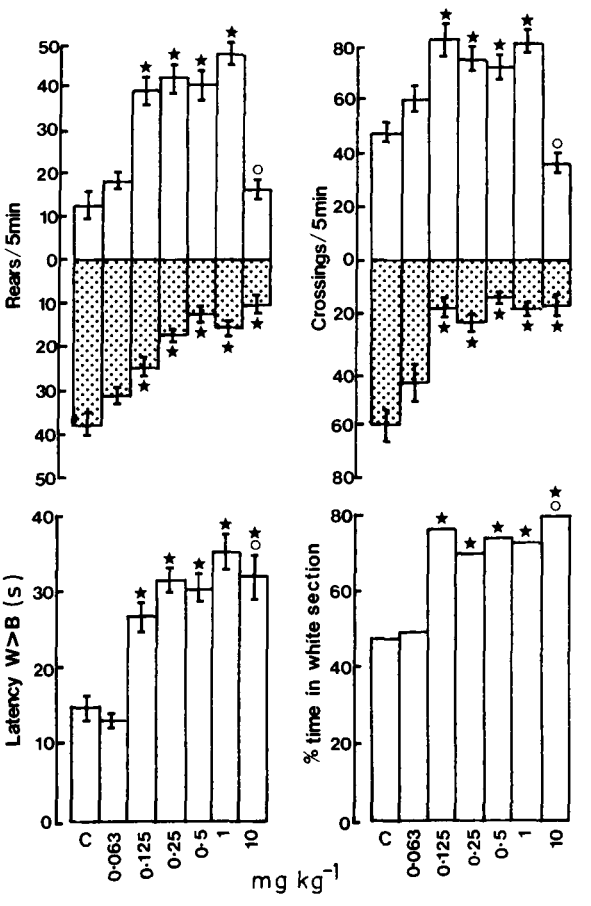

FIG. 2. Disinhibitory effects of diazepam in the light/dark discrimination test in the mouse. Mice were tested singly in an open-topped box, three-fifths painted white and brightly illuminated (white section) and partitioned (with an interconnecting door) from the remainder of the box which was painted black and illuminated with red light (dark section). The floor of each section was lined into 9 cm squares. Behavioural changes in rearing (rears), line crossings (crossings), latency of movement from the white (W) to the black (B) section (after first placement into the white area) and % time spent in the white area were recorded. In the upper set of histograms, hatched columns indicate data for the dark area, open columns for the light area. Data obtained from control (C) and drug treated mice were analysed using single factor analysis of variance, and Dunnett's *t*-test. Significant increases/decreases in responding are indicated as $*P<0{\cdot}05$–$P<0{\cdot}001$. n = 5. Vertical bars indicate s.e.m.s; s.e.m.s were less than 13·5% on original data for calculation of % time in white section. °sedation.

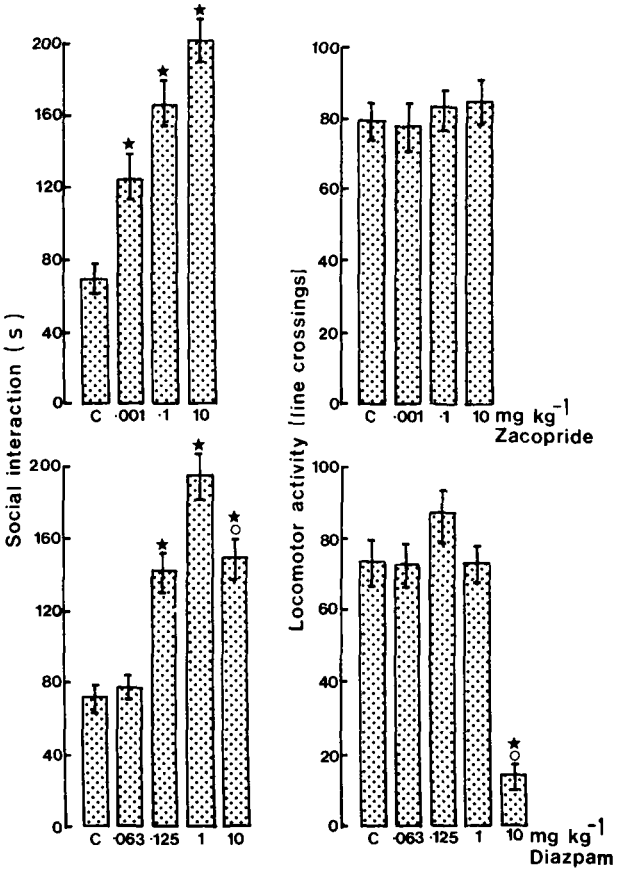

FIG. 3. Disinhibitory effects of zacopride and diazepam in the social interaction test in the rat. The time spent in active social interaction between pairs of rats was recorded during a 10 min period under the experimental conditions of high light and unfamiliarity of rats with each other. Crossings of lines marked on the test box floor were also noted. Data obtained in control (C) and drug treated rats was analysed by single-factor analysis of variance followed by Dunnett's *t*-test. n = 6. S.e.m.s given. Significant increases/decreases in responding are indicated $*P<0{\cdot}05$–$P<0{\cdot}01$). °sedation.

change to an increased exploratory activity in the white section and decreased in the black is not observed following the administration of other types of psychopharmacological agents (Costall et al 1987c; see also Crawley 1981).

Influence on rat social interaction and exploratory behaviour

In the rat social interaction test, performed under high light/unfamiliar conditions (see File 1980), social interaction of naive rats was at a low level, amounting to some 70 s per 10 min test period. Social interaction was enhanced by both diazepam and zacopride, the sniffing, crawling under and over partner, grooming, genital investigation and following partner being increased approximately 3-fold. The effects of zacopride (0·001–10 mg kg^{-1}) were not accompanied by any change in line crossings, indicating that zacopride does not influence locomotor activity. Diazepam (0·063–10 mg kg^{-1}) was approximately one hundred times less potent than zacopride and, as revealed by a decrease in line crossings, sedation occurred at the highest dose (Fig. 3).

Influence on behaviour of the marmoset exposed to a human threat situation

In response to a human threat the marmoset retreated from the front of the cage and spent only 25% of its time towards the front of the cage displaying about 10 postures/2 min during the 10 min period of observation. Zacopride (0·01 $\mu g\ kg^{-1}$) failed to antagonize the retreat from the cage front or the number of postures significantly. However, the higher dose of 0·1 $\mu g\ kg^{-1}$ zacopride markedly attenuated the retreat and reduced the posturing by 65%; the higher dose of 1·0 $\mu g\ kg^{-1}$ was even more effective, the marmosets remaining alert and active. Diazepam (10 $\mu g\ kg^{-1}$) also antagonized the effect of the human threat but the use of doses higher than 25 $\mu g\ kg^{-1}$ was precluded by the induction of sedation (Fig. 4).

Discussion

In the rodent and primate models described, both diazepam and zacopride were shown to modify behaviour in a manner consistent with an anxiolytic profile of action. Sulpiride has also

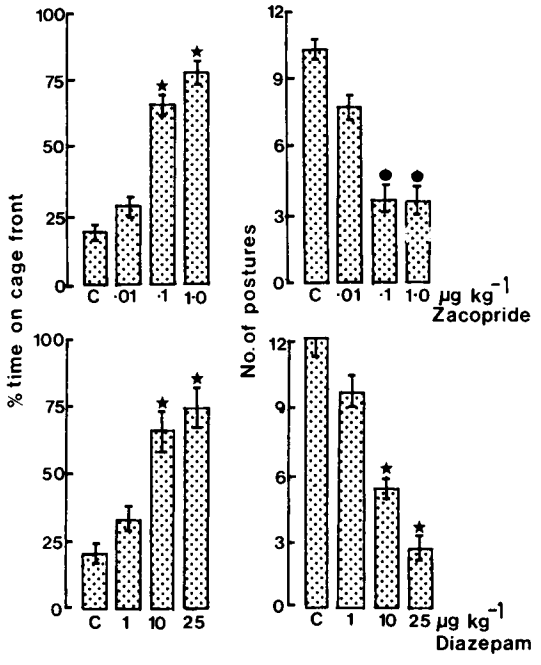

FIG. 4. Effect of zacopride and diazepam on the behavioural changes provoked by a human threat in the common marmoset. Behavioural changes were measured over a 2 min period. The Figure shows changes in posturing (s.e.m.s given) and % of time spent on the cage front (s.e.m.s on % of time calculated from original data) n = 4. Data obtained for control (C) and drug-treated animals was analysed by one-way analysis of variance followed by Dunnett's *t*-test. Significant increases/decreases in responding are indicated $*P<0{\cdot}05$–$P<0{\cdot}001$.

been shown to have an anxiolytic action in the rodent and primate models (Costall et al 1987c, unpublished data) although zacopride is at least two orders of magnitude more potent than either sulpiride or diazepam. Whilst it is generally considered that the benzodiazepines may exert their effects through the benzodiazepine-GABA receptor complex (Haefely 1983), there is no indication that the substituted benzamides can interact at the benzodiazepine recognition site. However, there is increasing evidence to support a role for 5-hydroxytryptamine in animal models which are considered to be sensitive to the detection of anxiolytic activity (see review by Gardner 1986). Thus, drugs acting as agonists/antagonists at the 5-HT_{1A} receptors, e.g. buspirone, ipsapirone, or 5-HT_3 receptors, e.g. ICS 205–930, GR38032F have been shown to be active in several models which are believed to be sensitive to anxiolytic activity (Dourish et al 1986; Eison et al 1986; Costall et al 1987b; Tyers et al 1987), and zacopride has been shown to be a potent 5-HT_3 antagonist (Smith et al 1988). Therefore, the profile of behavioural change caused by zacopride, which is consistent with changes caused by known anxiolytic agents, may reflect a 5-HT_3 receptor antagonism. It is suggested that this profile of action, already recorded for tropanyl and carbazolone derivatives, may also be found in the substituted benzamide series.

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