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Anxiolytic-like effects of DAIZAC, a selective high-affinity 5-HT_3 receptor antagonist, in the mouse elevated plus-maze

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Abstract

Behavioral effects of desamino-3-iodozacopride (DAIZAC) [(*S*)-5-chloro-3-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide], a selective high-affinity 5-HT_3 receptor antagonist (K_D 0.14 nM), were evaluated in the mouse elevated plus-maze using the anxiolytic benzodiazepine, diazepam, as a positive control. DAIZAC treatment produced a significant dose-related increase in the time spent in the open arm. The increased total time in the open arm resulted from a significant dose-dependent increase in the number of entries into that arm. The minimum dose of DAIZAC associated with a statistically significant increase in entries and time spent in the open arm was 0.05 mg/kg ip, consistent with its high affinity for the 5-HT_3 receptor. DAIZAC did not affect the amount of time spent in the open arm after each entry. Thus, DAIZAC reduced apparent avoidance of the open arm when the animal was in the central compartment, without affecting active avoidance of that arm when the animal was in the exposed condition. The increase in the open-arm entries was accompanied by a corresponding reduction in the number of entries into the closed arm with a consequent reduction in the time spent in the closed arm. The time spent in the closed arm after each entry was not altered by DAIZAC administration. As such, the sole apparent effect of DAIZAC was to alter the choice of arm to enter when the animal was in the central compartment. Diazepam also significantly increased total time in the open arm; however, the increase was not attributable to a single behavioral factor. The anxiolytic-like effects of DAIZAC reached maximum by 20–30 min and returned to baseline levels by 90 min. Ex vivo binding studies found that levels of DAIZAC-like activity assayed in brains of mice 25 min after DAIZAC injection were significantly correlated with the behavioral parameters associated with anxiolysis. These results indicate that DAIZAC produces dose-dependent anxiolytic-like behavioral changes in the mouse elevated plus-maze that are correlated with brain DAIZAC-like activity.

Keywords: DAIZAC; 5-HT_3 receptor antagonist; Diazepam; Anxiolytics; Elevated plus-maze; Mice

1. Introduction

Benzodiazepines have dominated the treatment of anxiety disorders for over four decades. While they are highly effective for treatment of generalized anxiety disorder (Ballenger, 1999), their significant abuse potential and side-effect profile (Uhlenhuth et al., 1999; Woods et al., 1992) has led to a search for nonbenzodiazepine anxiolytic agents. Animal models have been successful in identifying drugs effective in treating generalized anxiety disorder. 5-HT_3 antagonists represent an attractive alternative to benzodiazepines because they do not produce sedation, motor impairment, or amnesia, nor do they have addictive properties or produce significant withdrawal symptoms after discontinuation of chronic administration in animal studies [for reviews, Costall and Naylor, 1991, 1992; Costall et al., 1989; Gao and Cutler, 1992; Greenshaw and Silverstone, 1997; Hogg, 1996; Jones et al., 1988, 1993; Mos et al., 1989; Olivier et al., 2000; Rodgers et al., 1992; Wettstein, 1992]. 5-HT_3 antagonists have been reported to be anxiolytic in primate models of anxiety (Costall et al., 1988, 1993; Jones et al., 1988; Piper et al., 1991); however, there have been conflicting findings for efficacy of 5-HT_3 antagonists in murine models of anxiety (Andrew and File, 1993a; Artaiz et al.,

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1995; Barnes et al., 1990; File and Johnston, 1989; Filip et al., 1992; Griebel et al., 1997; Morinan, 1989; Mos et al., 1989; Piper et al., 1988; Wright et al., 1992).

Preliminary clinical studies of 5-HT_3 antagonists have shown some promise. We have reported preliminary evidence that the 5-HT_3 antagonist, ondansetron, may be effective in reducing the symptoms of obsessive–compulsive disorder (OCD) (Hewlett et al., 1999a). This is of interest because classic benzodiazepines appear to be mostly ineffective in treating this disorder (Greist and Jefferson, 1998; Hewlett, 2000). Another 5-HT_3 antagonist, tropisetron, was found to have beneficial effects in the treatment of generalized anxiety disorder (Lecrubier et al., 1993). Ondansetron has also been shown to reduce the intensity of panic attacks (Schneier et al., 1996), and reduce CCK-induced panic symptoms in panic disorder patients (Depot et al., 1999); however, contradictory results for ondansetron in panic disorder have also been reported (McCann et al., 1997), and as yet, no 5-HT_3 antagonists are marketed for the treatment of any anxiety disorder.

With the intent of developing selective high affinity 5-HT_3 receptor antagonists as tools for studying the role of 5-HT receptors in the CNS functioning and in animal behavior, we have synthesized and tested a series of novel zacopride analogs that act as 5-HT_3 receptor antagonists. Among them, desamino-3-iodozacopride (DAIZAC; [(*S*)-5-chloro-3-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide]), was shown to have high affinity ($K_D \approx$ 0.14 nM) and selectivity (>1000-fold discrimination) for the 5-HT_3 receptor (Hewlett et al., 1999b; Mason et al., 1996). [^{125}I]DAIZAC binding to rat and mouse brain membranes is not significantly displaced by ligands having selective affinity for other 5-HT receptor subtypes, nor by ligands with high affinity for other neurotransmitter binding sites. Moreover, micromolar concentrations of unlabeled DAIZAC do not appreciably displace receptor-selective radiolabeled ligands from other serotonergic or nonserotonergic binding sites. We elected to test DAIZAC in the mouse elevated plus-maze model to determine: (1) the potential anxiolytic-like effects of DAIZAC; and (2) the relationship between the anxiolytic-like effects of DAIZAC and the blockade of 5-HT_3 receptors estimated by measuring the levels of DAIZAC in brain. To partially control for methodological factors, diazepam was used as a positive control in validating the model for anxiolytic-like behavioral effects. A preliminary report of the anxiolytic-like effects of DAIZAC have been presented at the Society for Neuroscience (Hewlett et al., 1998).

2. Materials and methods

2.1. Animals

The 191 male ICR mice weighing approximately 30 g (Harlan, Indianapolis, IN) were housed five per cage ($5'' \times 7'' \times 11''$) and maintained on a 12-h light/dark cycle (lights on 6:00–18:00 h) at 22°C with water and food available ad libitum. Animals were not handled for at least 7 days prior to experiments. The experimental protocol was approved by the Vanderbilt Institutional Animal Care and Use Committee and is in compliance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Unlabeled DAIZAC and radiolabeled [^{125}I]DAIZAC (specific activity 1400–1900 Ci/mmol) were prepared as previously reported (Mason et al., 1996). Diazepam was generously provided by Hoffmann La-Roche (Nutley, NJ). DAIZAC and diazepam were dissolved in 50–100 μl of 0.1 N HCl or polyethylene glycol, respectively. Both drugs were diluted with 0.9% saline, and injected intraperitoneally using a volume of 10 ml/kg body weight. For the time-course study, mice were injected with 0.5 mg/kg DAIZAC ip and were tested at 2.5, 5, 10, 20, 30, 60, 90, and 120 min postinjection. For dose–response studies, four doses of DAIZAC (0.005, 0.05, 0.5, and 5 mg/kg body weight) or diazepam (0.003, 0.03, 0.3, and 3 mg/kg body weight), and their respective vehicles were evaluated. The dose-dependent effects of DAIZAC and diazepam on behavior in the plus-maze were assessed 20 min and 30 min following intraperitoneal administration, respectively.

2.3. Automated elevated plus-maze test

The construction and use of the mouse elevated plus-maze employed has previously been described by Onaivi et al. (1990). Briefly, it consists of two open arms and two enclosed arms with walls 40 cm in height constructed of black Plexiglas. The arms were arranged in a plus (+) configuration, with the open and closed arms opposite each other. Two pair of photocells were strategically placed at the entrance and proximal arm of each of the open and closed arms to monitor entries and time spent in the open, closed, and central portions of the plus-maze as previously described by Onaivi et al. (1990, 1992). Entries were registered only when both photobeams were interrupted by the animal's body. The entire maze was elevated to a height of 45 cm from the floor. All testing took place between 9:00–12:00 a.m. Mice were allowed to adapt in a dimly illuminated room for 1 h prior to the test. Testing followed in a dimly lit condition with the experimenter present. Mice were placed in the center of the maze facing one of the open arms. The number of entries and the amount of time spent in the open arms, enclosed arms, and the central platform were automatically recorded by computer software over a 5-min tests period. The maze was cleaned with a 50% ethanol solution and dried by a stream of warm air between tests. The order of testing was randomized according to treatment group and each mouse was tested only once. The brains of all mice were immediately

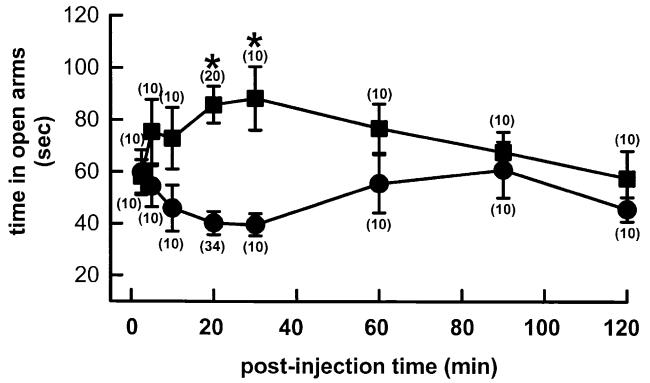

Fig. 1. Time course of the effect of DAIZAC on the time spent in the open arm of the mouse elevated plus-maze. Animals were tested 2.5, 5, 10, 20, 30, 60, 90, and 120 min postinjection following intraperitoneal administration of vehicle (●) or 0.5 mg/kg DAIZAC (■). Data are expressed as mean ± S.E.M. Animal numbers are indicated in parenthesis. * $P < .05$ compared to time-matched controls (two-way ANOVA followed by Dunnett's method).

removed after the behavioral test and stored at $-70°C$ until used for ex vivo binding (see below).

2.4. 5-HT_3 receptor binding activity in brain supernatants from DAIZAC-treated mice

Brains of DAIZAC and vehicle-treated mice, without cerebellum, were homogenized using a Brinkman polytron Model PT 3000 (15 s at 21,700 rpm) in 3 ml of assay buffer (50 mM HEPES, pH 7.4), containing 5 mM $CaCl_2$ and 2.4 mM $MgCl_2$. The homogenate was centrifuged at $10,000 \times g$ at 4°C for 15 min, and the supernatant was tested for its ability to inhibit [^{125}I]DAIZAC binding to brain membranes prepared from untreated mice.

Mouse brain membranes were prepared from frozen mouse brains (Pel Freeze Biologicals, Rogers, AR) as previously described (Hewlett et al., 1999b), and suspended in a final tissue concentration of 15 mg/ml. The membranes were incubated (22°C) in 0.1 nM [^{125}I]DAIZAC for 1 h together with either unlabeled DAIZAC at final concentrations ranging from 0.008 to 2.0 nM or with brain supernatants from DAIZAC-treated mice in dilutions from 1:25 to 1:400 in a total volume of 0.4 ml. Nonspecific binding was defined by 4.0 μM bemesetron. Bound and free [^{125}I]DAIZAC were separated by rapid filtration through No. 32 fiberglass filters (Schleicher & Schuell, Keene, NH), presoaked in 0.3% polyethylenimine for 10 min, using a Brandel M-24R cell harvester. Filters were washed three times for 10 s with 50 mM Na_2HPO_4 buffer (pH 7.4), and the radioactivity was measured by gamma spectrometry (Isomatic 4/600 HE, ICN Biomedic) at 80% efficiency. The amount of DAIZAC-like activity in the brain supernatants was estimated by comparing the displacement of [^{125}I]DAIZAC binding produced by the supernatant with the displacement produced by graded amounts of authentic DAIZAC. The term DAIZAC-like activity was adopted because ex vivo binding is not able to discriminate between the displacement of [^{125}I]DAIZAC binding produced by DAIZAC from that produced by DAIZAC metabolites or other substances. Some undiluted brain supernatants from saline-treated mice were found to displace [^{125}I]DAIZAC binding (equivalent to 1.9 ± 0.5 fmol/mg tissue). Therefore, binding studies were performed using dilutions at which supernatants from saline-treated rats had no effect on binding. DAIZAC-like activity was expressed as fmol DAIZAC per mg brain tissue.

2.5. Statistical analysis

Data were expressed as mean ± S.E.M. Two-way analysis of variance (ANOVA) was used to evaluate the effect of time on DAIZAC or DAIZAC vehicle administration. One-way ANOVA was used to evaluate the effect of dose on DAIZAC or diazepam administration. Statistical significance ($P < .05$) in the setting of multiple comparisons within each ANOVA were evaluated by Dunnett's method. Significance of individual differences from control conditions in behavioral measures were estimated using Fisher's Protected Least Squares Differences (FPLSD). Linear regression analysis was used to determine the correlation between brain DAIZAC-like activity and behavioral effects. Statistical significance was defined as $P < .05$.

3. Results

3.1. Time course of DAIZAC-induced behavioral effects

Two-way ANOVA of time in the open arm vs. post-injection testing interval (Fig. 1) showed that administration

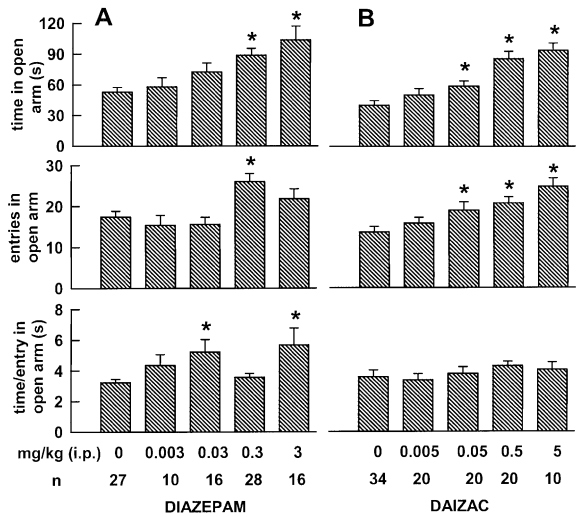

Fig. 2. Dose–response of time spent in the open arm, number of entries, and time per entry in the mouse elevated plus-maze after intraperitoneal administration of vehicle and four doses of diazepam (A) or DAIZAC (B). Data are expressed as mean ± S.E.M. * $P < .05$ compared to control groups (one-way ANOVA followed by Dunnett's method).

of DAIZAC (0.5 mg/kg ip) produced a significant increase in time spent in the open arm compared to vehicle-treated mice [$F(1,7) = 25.56$, $P < .0001$] in a postinjection interval-dependent fashion [$F(1,7) = 2.36$, $P = .025$]. A statistically significant increase in open-arm time was first apparent in mice tested 20 min after DAIZAC administration (FPLSD, $P < .0001$). Time spent in the open arm reached maximum by 20–30 min and returned to baseline levels by 90 min. Therefore, a 20-min postinjection time interval was used for dose–response testing of the effects of DAIZAC on behavior in the plus-maze, with a consequent 25-min interval between injection and sacrifice for measurements of brain DAIZAC-like activity.

3.2. Dose–response of DAIZAC and diazepam

The dose–response curves of the effects of diazepam and DAIZAC on open and closed arm behavior in the maze are presented in Figs. 2 and 3, respectively. One-way ANOVA revealed that DAIZAC significantly increased the time spent in the open arm [$F(4,99) = 14.12$, $P = .001$] in a dose-dependent manner (Fig. 2B). The minimum dose inducing a statistically significant increase in open arm time was 0.05 mg/kg (FPLSD, $P = .015$). The increased time spent in the open arm produced by DAIZAC was solely a product of a significant increase in the number of entries into the open arm [$F(4,99) = 5.83$, $P = .001$]. There was no change in the average time spent in the open arm after each entry [$F(4,99) = 0.67$, $P = .617$]. Some investigators have employed the terms [open-arm time]/[open-arm time + closed-arm time] (%OAT) and [open-arm entries/total arm entries] (%OAE) as a measures of anxiolytic-like activity in the plus-maze. DAIZAC administration was associated with a dose-related increase in both %OAT [$F(4,99) = 14.94$, $P < .0001$] and %OAE [$F(4,99) = 11.06$, $P < .0001$].

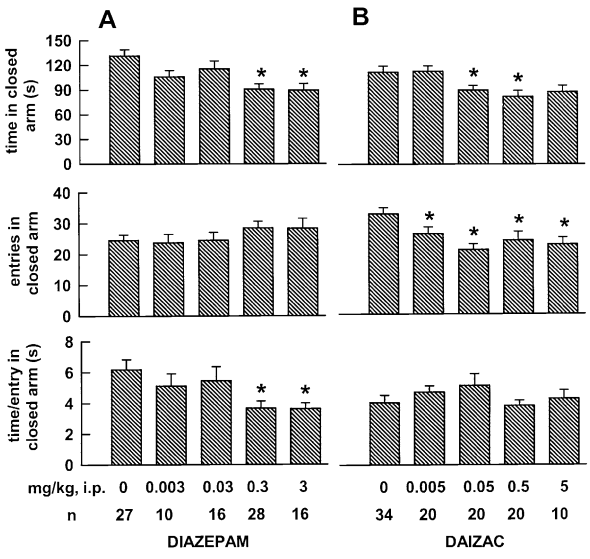

Fig. 3. Dose–response of time spent in the closed arm, number of entries, and time per entry in the mouse elevated plus-maze after intraperitoneal administration of vehicle and four doses of diazepam (A) or DAIZAC (B). Data are expressed as mean ± S.E.M. * $P < .05$ compared to control groups (one-way ANOVA followed by Dunnett's method).

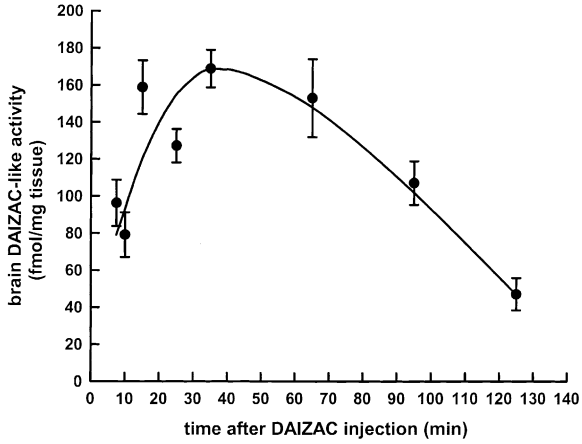

Fig. 4. Time course of DAIZAC-like activity in mouse brain following DAIZAC administration (0.5 mg/kg ip). Data represent mean ± S.E.M. of 5–10 determinations.

DAIZAC also produced a dose-dependent reduction in the time spent in the closed arm of the maze [$F(4,99) = 3.71$, $P = .008$; Fig. 3B]. As with open-arm behavior, the reduction in total closed-arm time resulted solely from a decrease in the number of closed-arm entries [$F(4,99) = 4.99$, $P = .001$; Fig. 3B], and not from any change in the average time spent in that arm after each entry [$F(4,99) = 1.54$, $P = .20$]. The effect of DAIZAC on entries into the two arms was to change the ratio of open to closed entries ($0.42 \rightarrow 1.11$). DAIZAC did not alter the total number of entries into the two arms [$F(4,99) = 1.09$, $P = .368$], but did induce a dose-related reduction in the amount of time spent in the central area that was barely significant [$F(4,99) = 2.504$, $P = .047$; data not shown]. Only the highest dose of DAIZAC (5 mg/kg) induced a statistically significant change in this measure (FPLSD, $P = .011$).

As expected, one-way ANOVA also demonstrated that the positive control, diazepam, produced a significant dose-dependent increase in the time spent in the open arm [$F(4,92) = 6.85$, $P < .0001$; Fig. 2A]. In this model, with the exception of the 0.3 mg/kg dose, the increase was a solely a result of increased time spent in the open arm after each entry [$F(4,99) = 3.56$, $P = .0095$]. Diazepam did not significantly change the number of entries into the open arm at other doses. In stark contrast, at 0.3 mg/kg dose, diazepam induced a significant increase in the number of open-arm entries (FPLSD, $P = .0003$), but no change in the time after entries into the open arm (FPLSD, $P = .62$). %OAT was significantly elevated by diazepam [$F(4,92) = 7.75$, $P = .001$]; however, %OAE was not affected [$F(4,92) = 1.39$, $P = .24$].

Diazepam also produced a dose-dependent decrease in the time spent in the closed arm [$F(4,92) = 6.34$, $P = .0002$; Fig. 3A]. In this case, the number of entries into the closed arm was unaffected by diazepam administration [$F(4,92) = 0.79$, $P = .54$], and the decrease in closed arm time was completely accounted for by the decrease in the time spent in that arm after each entry [$F(4,92) = 6.34$, $P = .0002$]. The total number of arm entries increased with diazepam administration [$F(4,92) = 5.87$, $P = .0003$]; however, only the 0.3 mg/kg dose produced a statistically significant change (FPLSD, $P = .0003$) resulting from the increase in open-arm entries at this dose. There was no change in the time spent in the center of the plus-maze [$F(4,92) = 1.50$, $P = .21$].

3.3. Brain DAIZAC-like activity

Mice treated with DAIZAC (0.5 mg/kg ip) and sacrificed at different postinjection times (7.5–125 min) were used for analysis of the time course of DAIZAC-like activity in brain (Fig. 4). Fifteen minutes after DAIZAC injection, the brain DAIZAC-like activity was 158 ± 3 fmol/mg tissue, and reached its peak level (169 ± 10 fmol/mg tissue) at 35 min. DAIZAC elimination from brain following peak levels was approximately linear over the indicated time interval and reached half the maximal value at approximately 100 min after injection. The DAIZAC-like activity in brain supernatants was significantly correlated with both the number of entries into the open arm ($r = .451$, $P < .01$) and the time spent in the open arm ($r = .586$, $P < .001$; Fig. 5).

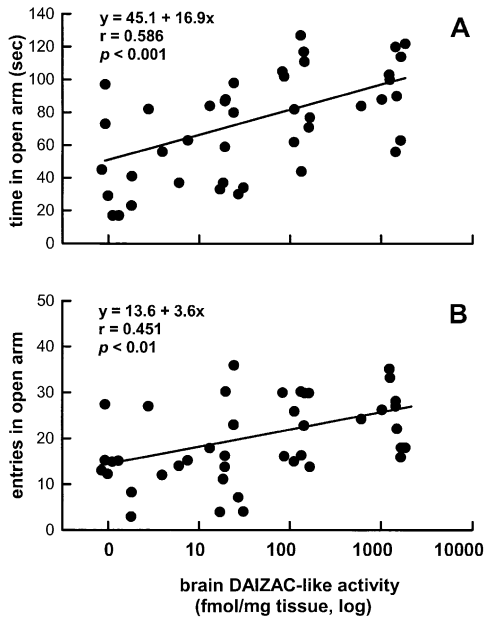

Fig. 5. Correlation of DAIZAC-like activity in mouse brain and the time spent in the open arm (A) and the number of open-arm entries (B) in the plus maze. Animals were tested 20 min postinjection and sacrificed after the test.

4. Discussion

At least nine murine studies using the elevated plus-maze model (Andrews and File, 1993b; Artaiz et al., 1995; Blackburn et al., 1993; Costall and Naylor, 1991; Costall et al., 1989, 1993; Dunn et al., 1991; Filip et al., 1992; Piper et al., 1988) have demonstrated anxiolytic-like actions of 5-HT_3 receptor antagonists. Consistent with these findings, one study using this model found anxiogenic-like activity in rats treated with the 5-HT_3 receptor *agonist*, mCPBG, after 21 days of vehicle injections (Andrew and File, 1993). There have also been conflicting findings. At least four elevated plus-maze studies have found no anxiolytic effects (File and Johnston, 1989; Griebel et al., 1997; Piper et al., 1988; Wright et al., 1992), and three studies found anxiolytic effects for some 5-HT_3 antagonists, but not for others (Artaiz et al., 1995; Filip et al., 1992; Pollack and Gould, 1996). Numerous studies using murine black–white box (Artaiz et al., 1995; Barnes et al., 1990; Bill et al., 1992; Costall et al., 1988, 1993; Eglen et al., 1994; Gao and Cutler, 1992; Jones et al., 1988; Kilfoil et al., 1989; Young and Johnson, 1991a,b) and social-interaction (Costall et al., 1988, 1993; Gao and Cutler, 1992) models of anxiety have also found anxiolytic-like activity for 5-HT_3 antagonists; however, negative findings have also been reported in these models as well (Andrew and File, 1993a; Barnes et al., 1990; File and Johnston, 1989; Morinan, 1989; Mos et al., 1989), and two studies have actually found anxiogenic-like properties for zacopride in the social interaction model (Andrew and File, 1993a; Barnes et al., 1990). Dosing may have been a factor in some of the negative findings. Few of the negative studies employed compounds having high affinity ($K_i < 1$ nM) and selectivity for the 5-HT_3 receptor. Additionally, many methodological factors are known to affect outcomes in these models. Such factors include age, species and strain differences, circadian factors, and the nature of the behavioral measurements recorded (Griebel et al., 2000; Rodgers et al., 1992). Physical and procedural variables such as manipulation of the animals prior to testing, prior exposure to novel environments, and presence of the experimenter during testing have been reported to alter, or even to abolish the anxiolytic-like effects of active compounds, including 5-HT_3 antagonists (Andrew and File, 1993a; Rodgers and Shepherd, 1993).

The present study suggests that DAIZAC induces anxiolytic-like changes in behavior in the mouse elevated plus-maze. The inference of anxiolytic activity in this automated plus-maze model derives from a vast body of literature relating changes in open arm behavior in different elevated plus-maze paradigms to anxiolysis (for review, see Hogg, 1996), and from previous studies of anxiolytic agents using this automated monitoring system (Davies et al., 1994; Onaivi et al., 1990, 1992, 1994, 1995; Salonen et al., 1992). The inference is further supported by the finding that DAIZAC-induced changes in anxiolytic-like behavior equivalent in magnitude to those produced by our positive control, diazepam.

DAIZAC increased both the time in the open arm and the number of entries into the open arm. It is unlikely that these changes were solely a consequence of increased locomotor behavior since the total number of arm entries did not increase as one might expect if DAIZAC had simply induced more linear movement. Recent studies have suggested that the number of closed-arm entries may be a better measure of locomotor activity (Rodgers and Johnson, 1995). If so, DAIZAC increased open-arm entries in the setting of a decreased locomotor tendency, as inferred from the reduced number of closed-arm entries in this condition. Changes in open-arm entries and open-arm time were significantly correlated with DAIZAC-like activity in mouse brain. Given the high selectivity of DAIZAC for the 5-HT_3 receptor relative to other CNS receptors and transporter sites, the correlations suggest that the anxiolytic-like actions of DAIZAC were mediated through selective blockade of 5-HT_3 receptors in brain.

While diazepam and DAIZAC induced similar changes in the time spent in each arm, there were behavioral differences in the manner by which these changes occurred. DAIZAC's primary effect was on the choice of arm to enter when the animal ventured from the central compartment, altering only the relative tendency to enter the arms from a preponderance of closed-arm entries in the control condition, to an approximately equal number of entries into the two arms with increasing doses of the drug. In contrast, increased total open-arm time following diazepam administration involved a more complex interaction of changes in open-arm entries and average time spent in the open arm after each entry. The behavioral effects of the two drugs were more clearly divergent in the manner by which they reduced total closed-arm time. Unlike DAIZAC, diazepam only decreased time spent in the closed arm after entry, and had no effect on the number of entries into that arm. These differences between the two drugs could relate to different anxiolytic mechanisms of action, or they could relate to extraneous factors unrelated to anxiolysis, such as differences in time course of action, differential dose-related sedative effects, or unique interactions of these compounds in this particular species of mice.

The distinctive behavioral changes induced by DAIZAC raise questions regarding motivational components associated with these behaviors. To the extent that time spent in the open arm after entry is a reflection of situational aversion to the exposed condition, DAIZAC did not appear to affect the tendency to leave the arm once the animal was in the exposed condition. That is, DAIZAC did not affect apparent active avoidance of the open arm once the animal was already in it. In contrast, DAIZAC did appear to reduce the animal's tendency to avoid the open arm when the animal was in the central compartment, as reflected by the increased choice of entry into that arm. Thus, DAIZAC appeared to reduce passive avoidance of the open arm when the animal was in the central compartment without affecting active avoidance of that arm when the animal was in the exposed condition.

A reduced tendency to avoid entry into the open arm is consistent with an altered perception of anticipated risk associated with entering that arm. If highly selective 5-HT_3 antagonists can alter perceived risks associated with behavioral acts, this could have important implications for the treatment of anxiety disorders in man. Motivational elements associated with avoidance behaviors in the plus-maze — risk-perception and situational aversion, have important roles in the different clinical presentations of anxiety disorders. An aberrant, heightened perception of risk is a striking feature in the psychopathology of OCD. It is tempting to note that the apparent ability of DAIZAC to reduce presumed risk-related avoidance of entry into the open arm in this model parallels the efficacy of the 5-HT_3 antagonist, ondansetron, in reducing pathological risk-related perceptions in OCD. In noting such parallels, however, it is important to point out that the data from the automated plus-maze model in these studies do not include ethological observations of animals during the drug trials. Thus, it is not known how DAIZAC affects putative "risk assessment" behaviors such as stretch-attend postures or aborted arm entry attempts in this model, or how it might alter stereotypic, locomotor, or exploratory behaviors in the maze. Ethological observations in this model will be critical in future studies to determine the extent to which changes in specific behavioral patterns within the maze might be associated with the observed anxiolytic-like behavioral changes induced by DAIZAC. Additionally, further studies with potent, selective, and long-lived 5-HT_3 antagonists, both in animal models and in human clinical populations, may elucidate common motivational elements of behavioral change induced by 5-HT_3 antagonists.

In summary, DAIZAC, a potent and highly selective 5-HT_3 receptor antagonist, was shown to produce anxiolytic-like behavior in the mouse elevated plus-maze. These effects were significantly correlated with whole-brain DAIZAC-like activity measured by ex vivo binding. The dose-dependent increase in the amount of time spent in the open arm produced by DAIZAC differed from that of diazepam in that it was solely related to changes in the choice of which arm to enter, and not to changes in time spent in either arm after entry. These differences may emanate from differential pharmacological effects on motivational components involved in plus-maze behavior.

Acknowledgments

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