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TDIQ (5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinoline) exhibits anxiolytic-like activity in a marble-burying assay in mice

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Abstract

Numerous studies have suggested that the central α_2-adrenergic receptor system may exert an important role in some types of human anxiety. The anxiolytic-like activity and potential side effect-like activities of the novel and purported α_2-adrenergic compound TDIQ (5,6,7,8-1,3-dioxolo[4,5-*g*] isoquinoline) were compared to those of the anxiolytic drugs diazepam and buspirone, and the nonselective α_2-adrenergic agent clonidine. Anxiolytic-like behavior was assessed in an object (marble)-burying assay, a selective test for the evaluation of known anxiolytics and identification of putative antianxiety compounds, that used mice housed either alone or in groups (5/cage). The rodents' antianxiety-like effect was defined as dose-related increases in the number of marbles that remained uncovered in their bedding material without concomitant disruption of their motor activities. Rotarod and inclined screen procedures were employed as potential indicators of side effects. An additional test monitored the heart rate (HR) and blood pressure (BP) of mice after the intravenous (IV) administration of doses of TDIQ. The reference compounds inhibited marble-burying behavior in a dose-related manner and produced various degrees of impairment in the side effect tests. TDIQ also inhibited object burying and displayed a wide separation between doses that produced anxiolytic-like activity and doses that produced some, if any, disruption of coordinated movement and/or motor activity. Moreover, the IV administration of TDIQ, up to 10 mg/kg, produced negligible effects on the HR and BP of mice. TDIQ could be a lead candidate for a new type of structural compound in the treatment of certain forms of anxiety.

Keywords: Noradrenergic; Alzheimer's disease; Dementia; Drug abuse; Insomnia; Norepinephrine; Obsessive–compulsive disorder (OCD); Panic disorder; Post-traumatic stress disorder (PTSD); Premenstrual syndrome (PMS); Sundowning

1. Introduction

Several lines of evidence indicate the possible involvement of α_2-noradrenergic receptors in the etiology and treatment of anxiety. It is suspected that excessive activity, and consequent dysregulation, of central α_2-noradrenergic receptors may be involved, at least in part, in anxiety disorders such as panic disorder, post-traumatic stress disorder (PTSD), obsessive–compulsive disorder (OCD), psychological problems associated with premenstrual syndrome (PMS), insomnia that may be related to anxiety, behavioral agitation and confusion that is linked to dementia (i.e. "sundowning"), and symptoms of anxiety that arise from drug abuse (e.g., opioid, alcohol, or cocaine) withdrawal syndromes (e.g., Berridge and Waterhouse, 2003; Bremner et al., 1996; Heninger et al., 1988; Hollander et al., 1991; McDougle et al., 1994; Nilsson et al., 1985).

Noradrenergic neurons are regulated primarily by neuronal feedback inhibition that is controlled by presynaptic α_2-noradrenergic autoreceptors; postsynaptic α_2-noradrenergic receptors also exist. The activation of these autoreceptors, by norepinephrine or by norepinephrine autoreceptor agonists, exerts an inhibitory influence on the firing rate of neurons and decreases the release of norepinephrine in the locus coeruleus, the principal location of α_2-noradrenergic receptors, and other areas throughout the brain. Conversely, the antagonism of these receptors can produce an enhancement in the firing rate of neurons and an increase in the release of norepinephrine

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(e.g., Docherty, 1998). α_2-Noradrenergic receptors may be involved in the mediation of an individual's state of arousal and, perhaps, symptoms of anxiety (Redmond and Huang, 1979). If some forms of anxiety do result from overactivity of brain α_2-noradrenergic systems, then drugs that stimulate α_2-noradrenergic autoreceptors or block α_2-noradrenergic postsynaptic receptors, and consequently decrease noradrenergic function, might exhibit antianxiety effects. For example, clonidine, an imidazoline derivative, is a nonselective α_2-noradrenergic receptor agonist and has been used clinically in the treatment of anxiety symptoms, including those associated with opioid and alcohol withdrawal syndromes, hypertension, and as an analgesic (Carnwath and Hardman, 1998; Hoehn-Saric et al., 1981; McDougle et al., 1994). Unfortunately, the use of clonidine is quite limited due to its frequently reported side effects of dry mouth, drowsiness, dizziness, nausea, hypotension and, in some males, sexual dysfunction (Gavras et al., 2001; Guyenet, 1997). Although these side effects are often assumed to be the result of α_2-adrenergic activity, it should be noted that radioligand binding studies have consistently shown that clonidine is only slightly selective for α_2-adrenergic receptors over α_1-adrenergic and imidazoline (I_1) sites and also shows marked (albeit lower) affinity at serotonin 5-HT_{1A} receptors (e.g., Millan et al., 2000a,b; Newman-Tancredi et al., 1998). Other traditionally employed α_2-adrenergic agents such as yohimbine, idazoxan, rauwolscine, guanfacine, and guanabenz also exhibit marked affinity/activity at α_1-adrenergic, imidazoline, 5-HT_{1A} and/or 5-HT_{2B} receptors (e.g., Callado et al., 1996; Miralles et al., 1993; Newman-Tancredi et al., 1998; Wainscott et al., 1998). The possible interaction(s) of these "α_2-adrenergic agents" with those other receptors has the potential to confound the conclusion(s) of a definitive link(s) of α_2-adrenergic receptor involvement in various behaviors, pathologies, and/or side effects. It is likely that the lack of a selective α_2-adrenergic receptor agent(s) has hindered a more precise characterization of the clinical significance of these receptors. Consequently, the identification of such a compound might be quite useful, both as a pharmacological probe and, perhaps, as an agent with the potential for improved therapeutic value.

Recent reports have described some biochemical and behavioral characteristics of TDIQ (5,6,7,8-tetrahydo-1,3-dioxolol[4,5-*g*]isoquinoline) a putative α_2-noradrenergic compound (Glennon et al., 2002; Malmusi et al., 1996; Young and Glennon, 2002). This compound was examined at more than 30 different receptor sites and displayed very little or no affinity for the various subpopulations of dopamine (DA_1, rDA_2, rDA_3, rDA_4, DA_5), serotonin (5-HT_{1A}, r5-HT_{1B}, h5-HT_{1B}, h5-HT_{1D}, r5HT_{2A}, r5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_6, 5-HT_7), or muscarinic (M_1–M_5) receptors and did not bind at the norepinephrine, dopamine, or serotonin transporters. It also lacked affinity for phencyclidine (rPCP) benzodiazepine (rBZ), and α_{1A}-, α_{1B}-, β_1-, and β_2-adrenergic receptors (Glennon et al., 2002). TDIQ does exhibit high affinity for α_2-adrenergic (i.e. α_{2A} K_i=75 nM, α_{2B} K_i=95 nM, α_{2C} K_i=65 nM) receptors (Glennon et al., 2002).

Behaviorally, TDIQ does not depress or stimulate, from control level, the movement of rodents in a locomotor activity test (Malmusi et al., 1996). It does, however, readily serve as an effective stimulus in animals trained in a drug discrimination paradigm, a useful procedure to examine the central effect(s) of a compound (e.g., Young and Glennon, 1986). Rats can be trained to discriminate TDIQ from saline vehicle in a two-lever drug discrimination task. In tests of stimulus generalization (i.e. substitution, recognition) and stimulus antagonism (i.e. blockade), the effect of TDIQ proved difficult to characterize precisely (Glennon et al., 2002; Young and Glennon, 2002; Young et al., 2004). For example, asymmetric generalization occurred between TDIQ and cocaine: a TDIQ stimulus generalized to cocaine but a cocaine stimulus did not generalize to TDIQ (Young and Glennon, 2002: Young et al., 2004). In addition, the TDIQ stimulus was shown to partially generalize to the serotonin 5-HT_{1A} anxiolytic agent buspirone but did not substitute to the benzodiazepine anxiolytic medication diazepam (Glennon et al., 2002). The occurrence of partial generalization is difficult to interpret but it has been suggested that it may occur because there are pharmacological effects that are common to both the training drug and the challenge agent (e.g., Young and Glennon, 1986). That is, partial generalization may have occurred between TDIQ and buspirone because there is some "overlap" in their neurochemical mechanism(s) of action. Buspirone, however, appears to produce effects in the CNS that are quite complex. For example, it is believed to exert an effect(s) at both presynaptic and postsynaptic 5-HT_{1A} receptors. It also may function as a dopamine DA_2 receptor antagonist and as a receptor antagonist at central α_2-noradrenergic autoreceptors and postsynaptic receptors (e.g., Sharp et al., 1993). Moreover, the effects of buspirone at noradrenergic sites are thought to be exerted by 1-pyrimidinylpiperazine (1-PP), a major metabolite that reaches a concentration level in the brain that exceeds that of the parent compound (e.g., Blier et al., 1991). Possibly, the TDIQ discriminative stimulus might have partially generalized to buspirone because of an α_2-noradrenergic link between TDIQ and buspirone (perhaps via 1-PP). If this is the case, an enhanced or a reduced level of noradrenergic activity might have mediated the result. If TDIQ produces an increase in brain noradrenergic activity, then it may be expected to exhibit an anxiogenic-like effect in an animal model of anxiety. On the other hand, if TDIQ produces a reduction in brain noradrenergic activity, then an anxiolytic-like effect may occur in animals.

In order to address the latter question, the present study evaluated the pharmacological effects of TDIQ and reference compounds in an animal test procedure that used object burying by mice as an indication of anxiolytic-like activity (e.g., Broekkamp et al., 1986). In the test, mice are placed in cages (similar to their home cages) that contain glass marbles that are evenly distributed, along the walls, on top of a layer of bedding material. Under control conditions, rodents bury a substantial number (i.e. 65–75%) of the marbles. An anxiolytic-like effect is assumed from drug-induced decreases in marbles buried (or the reciprocal measure of increases in marbles left uncovered) without concomitant behavioral impairments in tests that typically are used as potential indicators of side effect liability such as locomotor, rotarod, and/or inclined screen assays (e.g., Malick, 1987). In addition, the anxiolytic-like potential and

putative side effect profile of the reference compounds and TDIQ were examined more extensively by testing them in mice that were housed either alone (1/cage) or in a group (5/cage). Such housing conditions have been shown to be an important determinant of behavior and it has been proposed that rodents that are housed singly might serve as a useful animal model of clinical anxiety (e.g., Parker and Morinan, 1986). In fact, previous studies have reported that rats housed alone exhibited indices of purported anxiogenic-like activity such as enhanced release of central norepinephrine, reduced GABA receptor function, decreased radioligand binding to brain benzodiazepine receptors, and reduced sensitivity (i.e. potency) to diazepam when the animals were tested in a social interaction test (e.g., Miachon et al., 1990; Weinstock et al., 1978; Wongwitdecha and Marsden, 1996).

In the present study, the pharmacological effect of TDIQ and the reference drugs diazepam, buspirone, and clonidine were examined in a marble-burying assay that employed mice housed either in a group or alone. The operational definition of anxiolytic-like activity was an increase from control level in the number of marbles that were left uncovered in an animal's bedding material. The compounds were also tested in a rotarod procedure and an inclined screen assay, two tests that are commonly used to measure drug-induced impairments of behavior (e.g., Malick, 1987). The latter procedures were performed to account for a non-specific drug-induced decrease in animals' motor activity that could potentially influence their performance (i.e. the number of marbles left uncovered). Finally, a separate test evaluated the heart rate (HR)/blood pressure (BP) of mice after intravenous (IV) administration of TDIQ. The latter measurements were obtained to control for the possibility that an α_2-adrenergic agent might be able to produce changes in an animal's cardiovascular function that could question the demonstration of an anxiolytic-like effect.

2. Materials and methods

2.1. Animals

Male ICR mice (Harlan Sprague Dawley Inc., Indianapolis, IN) weighing 25–34 g at the time of testing were used in the marble-burying, rotarod, and inclined screen tests. They were housed in a temperature- and humidity-controlled vivarium room under a standard 12:12 h dark/light cycle (lights on at 0700). Mice that were assigned to the marble-burying assay were tested once; mice assigned to the rotarod test were also evaluated in the inclined screen procedure (see below). Prior to testing, mice were housed either individually (1/cage) or in groups (5/cage) for 14–21 days in solid-bottomed plastic cages (38×22×15 cm) that contained wood shavings (Sani-Chips®, P.J. Murphy Forest Products, Montville, N.J.) for bedding. Animal bedding material was changed once per week. Food and water were available ad libitum. The experiments were conducted according to the standards set by the Institutional Animal Care and Use Committee (IACUC) of Virginia Commonwealth University and the NIH Guide for Care and Use of Laboratory Animals. The cardiovascular measurements of heart rate (HR) and blood pressure (BP) were obtained from male C57BL/6 mice (25–30 g) from Harlan Inc. (Indianapolis, IN). These animals were also kept under a 12:12 h dark/light cycle with food and water available ad libitum. The experiment was approved by the IACUC of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) of the National Institutes of Health (NIH).

2.2. Marble-burying

Animals were naïve to the test room and were transported from the vivarium to the test site for a 60- to 90-min acclimation period prior to the start of the experiment. Tests were performed between 1100 and 1730 h. Mice were placed individually into plastic cages that were identical to their home cage; group-housed mice (i.e. each 5 mice/group cage) were tested at the same time to avoid a potential confound of a within-cage order effect (e.g., Holson et al., 1991; Kask et al., 2001). The cage lid was a metal grid but no food or water was present. The cage contained a 10 mm layer (total dry weight=125 g) of Sani Chips® bedding and 24 glass marbles (Champion® #16002 Marbles: 1.5 cm diameter) evenly spaced against the walls (4×8×4×8) of the cage. A clean cage, fresh bedding, and clean marbles were used for each mouse. Animals were assigned to treatment groups according to a table of random numbers (Winer, 1962). Drug doses for the mice that were housed under each condition were as follows: diazepam (0.03, 0.30, 1.0, 3.0, 10.0, and 30.0 mg/kg for those housed in groups and 1.0, 3.0, 10.0, and 30.0 mg/kg for those housed alone), buspirone (1.0, 3.0, 10.0, and 30.0 mg/kg for those housed under each condition), clonidine (0.01, 0.03, and 0.30 mg/kg for those that were housed in groups and 0.01, 0.03, 0.30, and 1.0 mg/kg for those housed by themselves), and TDIQ (0.03, 0.30, 1.0, 3.0, 10.0, and 17.0 mg/kg for those that were housed in groups and 0.30, 0.60, 1.0, 3.0, 6.0, 10.0, and 17.0 mg/kg for those housed alone). The number of mice tested at each dose was 8–12. The mice were injected with either drug or saline vehicle 30 min before the test; during this time they waited in their home cage. Mice were then placed individually into a test cage and left undisturbed for 30 min. At the end of that period the number of marbles at least 2/3 buried/1/3 uncovered was recorded. The percent of marbles left uncovered (i.e. number left uncovered÷24×100) was used as an indication of anxiolytic-like activity. The experimenter was unaware of a mouse's treatment (i.e. drug or vehicle) until after the completion of the experiment. The dose–response effect of each drug was analyzed by analysis of variance (statistically significant F value set at $p \leq 0.05$) and followed by Dunnett's post-hoc comparison test ($p \leq 0.05$) to determine statistical significance between control group and each dose group.

2.3. Rotarod

The rotarod test was used to test an animal's motor coordination. The ability of a mouse to maintain balance on a rotating cylinder (rotating toward the mouse) was measured with a standard rotarod apparatus (Economex® Rota-Rod; Columbus Instruments, Columbus, OH). The cylinder was 3.8 cm in diameter, covered with textured rubber and rotated at a speed of 4 rpm. Mice were confined to a section of the cylinder approximately 9.0 cm long by

black Plexiglas dividers. Ninety minutes before drug or vehicle administration the mice were trained to stay on the rotarod over four successive 1 min trials. Those mice that remained on the rod for at least two consecutive 60-s periods (i.e. 120-s) were retested 30 min before drug administration; mice that did not stay on the rotating rod for 120 s were discarded from the experiment. Mice that were successful in the retesting session (i.e. one 60-s test) were assigned to a treatment group according to a table of random numbers (Winer, 1962) and then given an injection of either vehicle or test drug and returned to their home cage condition (i.e. either 1/cage or 5/cage). Drug doses for mice that were housed under each condition were as follows: diazepam (1.0, 3.0, and 10.0 mg/kg for those housed under each condition), buspirone (1.0, 3.0, 10.0, and 30.0 mg/kg for those housed under each condition), clonidine (0.01, 0.10, and 1.0 mg/kg for those housed under each condition), and TDIQ (1.0, 3.0, 6.0, 10.0, 17.0, 30.0, 60.0, 75.0, and 100.0 mg/kg for those housed in groups and 1.0, 3.0, 10.0, 30.0, and 75.0 mg/kg for those housed alone). Eight mice were tested at each dose. After 30 min, the mice were tested again on the rotarod for ≤60 s. A notation was made if an animal did not fall, or fell, from the rotating rod. If a mouse fell from the rod, the time it spent on the rotarod was noted (data recorded but not presented or subjected to statistical analysis). The percent of mice that fell (i.e. number of mice that fell ÷ 8 mice × 100) was recorded for the vehicle treatment and for each dose of compound. The control group was compared to each dose group by a *z*-test for the significance ($p \leq 0.05$; critical $z = \pm 1.96$) of difference between two proportions.

2.4. Inclined screen

The inclined screen (30° incline, 1/4 in. mesh screen) test was performed immediately following the rotarod test. In some instances, the dose–response curve for a compound was completed in the rotarod test but an additional dose(s) was (were) needed to complete the dose–response effect in the inclined screen assay. The additional drug doses for mice that were housed under each condition were as follows: diazepam (30.0, 35.0, 40.0, and 75.0 mg/kg for those housed in groups and 30.0, 50.0, and 75.0 mg/kg for those housed alone), buspirone (50.0 and 75.0 mg/kg for those housed in groups and 60.0 mg/kg for those housed singly), and clonidine (10.0, 17.0, and 30.0 mg/kg for those housed in groups and 10.0 and 17.0 mg/kg for those housed alone). Eight mice were tested at each dose. In this procedure, mice were placed on the lower one-third portion of the screen. Those mice that climbed to the top of the screen within 60 s were assigned a score of passing and those that did not were given a score of failing. The percent of mice that were impaired (i.e. number of mice that failed to reach the top of the inclined plane ÷ 8 mice × 100) was recorded for the vehicle treatment and for each dose of compound. The control group was compared to each dose group by a *z*-test for the significance ($p \leq 0.05$; critical $z = \pm 1.96$) of difference between two proportions.

2.5. Heart rate and blood pressure

Mice ($n = 10$/dose) were anesthetized with 60 mg/kg IP sodium pentobarbital, supplemented as needed to maintain stable anesthesia. Briefly, the jugular vein and the carotid artery were cannulated with PE-10 tubing filled with heparinized saline for intravenous TDIQ (0.10, 1.0, and 10.0 mg/kg) injections and monitoring blood pressure (BP) and heart rate (HR), respectively. The arterial cannula was connected to a pressure transducer and to the IOX Data Acquisition System (Emka Technologies, France). The baseline mean blood pressure (MBP) was 76.5 (±4.3) and heart rate (HR) was 579 (±29.3). The dose- and time-dependent effects of TDIQ on BP and HR were analyzed by analysis of variance (statistically significant *F* value set at $p \leq 0.05$). Further details of the procedure can be found in Pacher et al. (2004).

2.6. Data presentation and statistical comparisons between dose–response relationships

Anxiolytic-like activity was defined as the percent of marbles left uncovered in a mouse's bedding material (i.e. number of marbles left uncovered ÷ 24 × 100). Where a dose–response relationship occurred, an ED_{50} (effective dose 50%) dose was calculated by the method of Finney (1952). In the marble-burying assay, the ED_{50} dose represents the calculated drug dose where the mice would be expected to leave 50% of the marbles uncovered and 50% of the marbles buried. In the rotarod and inclined screen tests, the ED_{50} dose represents the expected drug dose where 50% of the mice would fall from the rotating rod or fail to reach the top of the inclined screen, respectively. Statistical comparisons of the dose–response relationships for each drug were performed by *F*-tests for simple effects with the probability (*p*) of statistical significance set at $p \leq 0.05$. Prior to statistical evaluation, dose values were subjected to log transformation and percent response data were normalized to account for different baseline values.

2.7. Drugs

Buspirone HCl and clonidine HCl were purchased from Sigma-Aldrich (St. Louis, MO) and diazepam (base) was a gift from Hoffman La Roche (Nutley, NJ). TDIQ HCl (5,6,7,8-tetrahydro-1,3-dioxolol[4,5-*g*]isoquinoline hydrochloride) was synthesized in the Department of Medicinal Chemistry, Virginia Commonwealth University. Doses of each compound, except for diazepam, refer to the weight of the salt; diazepam was weighed as its base. Each drug, except diazepam, was dissolved in 0.9% saline; diazepam was suspended in saline to which one drop of Tween 80 was added. Compounds were prepared fresh daily and intraperitoneal injections (10 ml/kg) were made 30 min prior to testing.

3. Results

3.1. Mice housed in groups

3.1.1. Anxiolytic-like activity

Fig. 1 shows that mice treated with diazepam ($F(6, 49) = 16.43$, $p < 0.0001$), buspirone ($F(4, 35) = 6.67$, $p < 0.0005$), clonidine ($F(3, 28) = 15.34$, $p < 0.0001$), and TDIQ ($F(6, 49) = 11.80$, $p < 0.0001$) exhibited dose-related anxiolytic-like activity as reflected by increases in the percent of marbles left uncovered in the marble-burying (MB) assay. Dunnett's post-hoc

comparison test revealed that the response of the control group of mice was statistically different from the groups of animals that received the following doses of diazepam (3, 10, and 30 mg/kg), buspirone (10 and 30 mg/kg), clonidine (0.03 and 0.30 mg/kg), and TDIQ (1, 3, 10, and 17 mg/kg). Table 1 provides a comparison of ED_{50} (mg/kg; 95% confidence limits) values; the order of potency to produce anxiolytic-like activity is clonidine > TDIQ ≥ diazepam > buspirone.

3.1.2. Rotarod (RR)

A total of 230 mice had to be tested to meet the demand for 184 mice (i.e. 2 groups of vehicle control mice and 21 doses of test compounds × 8 mice/group). Thus, 8 out of 10 mice succeeded in meeting the qualifying criterion required for testing in the RR test (see Materials and methods). The administration of diazepam, buspirone, and clonidine produced dose-related impairments of RR activity (Fig. 1). The *z*-tests revealed that the response of the control group was statistically different ($p < 0.05$) from the following doses of diazepam (10 mg/kg), buspirone (30 mg/kg), clonidine (1 mg/kg), and TDIQ (60 mg/kg). Their ED_{50} values are noted in Table 1; the order of potency to produce disruption of coordinated movement is clonidine > diazepam > buspirone. In contrast, the administration of TDIQ did not produce a marked effect at the highest doses tested: only 13% of mice exhibited incoordination at 75 or 100 mg/kg of TDIQ. It was noted (personal observation), however, that some (~60%) of the mice appeared to exhibit some signs of "sympathetic" activity (e.g., tremor, tachycardia) at 75 mg/kg and 100 mg/kg of TDIQ, but these effects did not seem to influence their performance in the RR and IS tests.

3.1.3. Inclined screen (IS)

The administration of diazepam (ED_{50} = 34.4 mg/kg) produced dose-related impairments in this procedure (Fig. 1 and Table 1). Buspirone and clonidine did not produce complete dose–response relationships: maximal percent impairments of 63% and 57% of mice occurred at 75 mg/kg of buspirone and 30 mg/kg of clonidine, respectively (Fig. 1 and Table 1). The *z*-tests revealed that the response of the control group was statistically different ($p < 0.05$) from the following doses of diazepam (35, 40, and 75 mg/kg), buspirone (50 and 75 mg/kg), and clonidine (30 mg/kg). In contrast, TDIQ produced a negligible effect: only 13% of mice were impaired at 100 mg/kg of TDIQ (Fig. 1 and Table 1).

3.1.4. Statistical comparisons between dose–response relationships

Where possible, statistical comparisons were performed between the dose–response relationships of the agents that inhibited marble-burying (MB) versus the dose–response functions that disrupted rotarod (RR) and inclined screen (IS) activities (Table 1). Statistical differences were noted between those dose–response relationships for the following compounds: diazepam (MB versus RR, $F(1, 5) = 9.58$, $p < 0.05$; MB versus IS, $F(1, 9) = 172.5$, $p < 0.05$), buspirone (MB versus RR, $F(1, 4) = 36.14$, $p < 0.05$), and clonidine (MB versus RR, $F(1, 2) = 40.81$, $p < 0.05$). Buspirone, clonidine and TDIQ did not produce complete dose–response functions in the IS and/or RR procedures (Fig. 1 and Table 1) and, therefore, appropriate statistical tests between MB versus RR and/or IS could not be determined (CND). In those instances, an estimated ratio was calculated between the highest test dose of the treatment in the side effect test, and the ED_{50} dose of the compound in the MB procedure (Table 1). Buspirone and clonidine produced

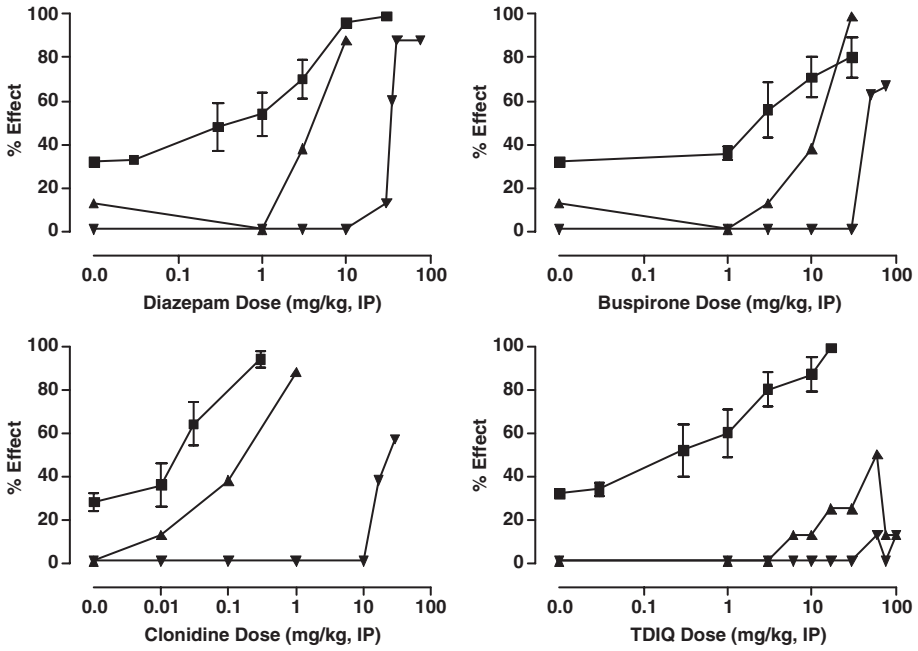

Fig. 1. Results of test agents on anxiolytic-like (i.e. percent of marbles that remained uncovered; ■—■), rotarod (i.e. percent of mice disrupted: ▲—▲), and inclined screen (i.e. percent of mice impaired; ▼—▼) activities in mice that were housed in groups (i.e. 5/cage) prior to testing. Ordinate: mean (with ±S.E.M. values in marble-burying assay) percent effect after the intraperitoneal administration (n = 8–12 mice at each point) of doses of each compound or 10 ml/kg of 0.9% saline (i.e. dose 0.0). Abscissa: drug doses plotted on a logarithmic scale.

Table 1
Comparison of test agents in mice that were housed in groups on marble-burying (MB), rotarod (RR), and inclined screen (IS) tests

Test agent	Marble-burying (MB)	Rotarod (RR)	Inclined screen (IS)	Ratio RR/MB	Ratio IS/MB
Diazepam	0.26 (0.07–0.96)	4.3 (2.3–8.3)	34.4 (30.8–38.4)	16.5*	32.3*
Buspirone	2.36 (0.57–9.68)	7.8 (3.5–17.5)	63% at 75 mg/kg	3.3*	CND>31
Clonidine	0.018 (0.005–0.07)	0.12 (0.02–0.89)	57% at 30 mg/kg	6.7*	CND>1600
TDIQ	0.20 (0.50–0.83)	13% at 100 mg/kg	13% at 100 mg/kg	CND>500	CND>500

Entry values are either the calculated ED_{50} dose (mg/kg dose with 95% confidence limits) or the highest tested dose (with percent of effect) of a compound in the procedure. Where possible, the ratios of ED_{50} doses for rotarod/marble-burying (RR/MB) and/or inclined screen/marble-burying (IS/MB) are presented; for convenience, an * is placed next to an ED_{50} dose to indicate statistical difference ($p \leq 0.05$) between the dose–response functions for the activity ratio. If a test compound did not produce a complete dose–response curve in a procedure, then it is noted that the dose–response statistical comparison and ED_{50} ratio comparison could not be determined (CND). In those instances, an estimated (i.e. >) ratio is given that is based on the highest test dose of the compound in the RR and/or IS tests and the ED_{50} dose of the agent in the MB assay.

ED_{50} doses (2.36 mg/kg and 0.018 mg/kg respectively) for anxiolytic-like activity that are at least 30 times and 1600 fold more potent than their highest test dose that produced some impairment in the IS test (i.e. 63% and 57% of mice were impaired at 75 mg/kg of buspirone and 30 mg/kg of clonidine respectively). TDIQ produced an ED_{50} dose (0.20 mg/kg) for anxiolytic-like behavior that is estimated to be at least 500 fold lower than its highest test dose that produced some impairment in the RR and IS tests (i.e. just 13% of mice were impaired in the latter tests at 100 mg/kg of TDIQ).

3.2. Mice housed alone

3.2.1. Anxiolytic-like activity

Fig. 2 indicates that mice treated with diazepam ($F(4, 43)=13.56$, $p<0.0001$), buspirone ($F(4, 47)=8.57$, $p<0.0001$), clonidine ($F(4, 35)=21.95$, $p<0.0001$), and TDIQ ($F(7, 58)=7.18$, $p<0.0001$) displayed dose-related anxiolytic-like activity as indicated by increases in the percent of marbles they left uncovered on top of their bedding material. Post-hoc (Dunnett Multiple Comparison Test) revealed that the response of the control group was statistically different from the following doses of diazepam (10 and 30 mg/kg), buspirone (30 mg/kg), clonidine (0.03, 0.30, and 1 mg/kg), and TDIQ (6, 10, and 17 mg/kg). Table 2 presents a comparison of ED_{50} values; the order of potency to produce an anxiolytic-like effect is clonidine>TDIQ>diazepam>buspirone.

3.2.2. Rotarod (RR)

A total of 230 mice had to be tested to meet the demand for 136 mice (i.e. 2 groups of vehicle control mice and 15 doses of test compounds×8 mice/group). Thus, only 6 out of 10 mice succeeded in meeting the qualifying criterion required for testing in the RR test. The administration of diazepam, buspirone, and clonidine produced dose-related impairments of RR activity (Fig. 2). The *z*-tests revealed that the response of the control group was statistically different ($p<0.05$) from the following doses of diazepam (3 and 10 mg/kg), buspirone (10 and 30 mg/kg), and clonidine (0.10 and 1 mg/kg). Their ED_{50} values are noted in Table 2; the order of potency to produce disruption of coordinated movement is clonidine>diazepam>buspirone. In comparison, the administration of TDIQ produced a limited effect in this test:

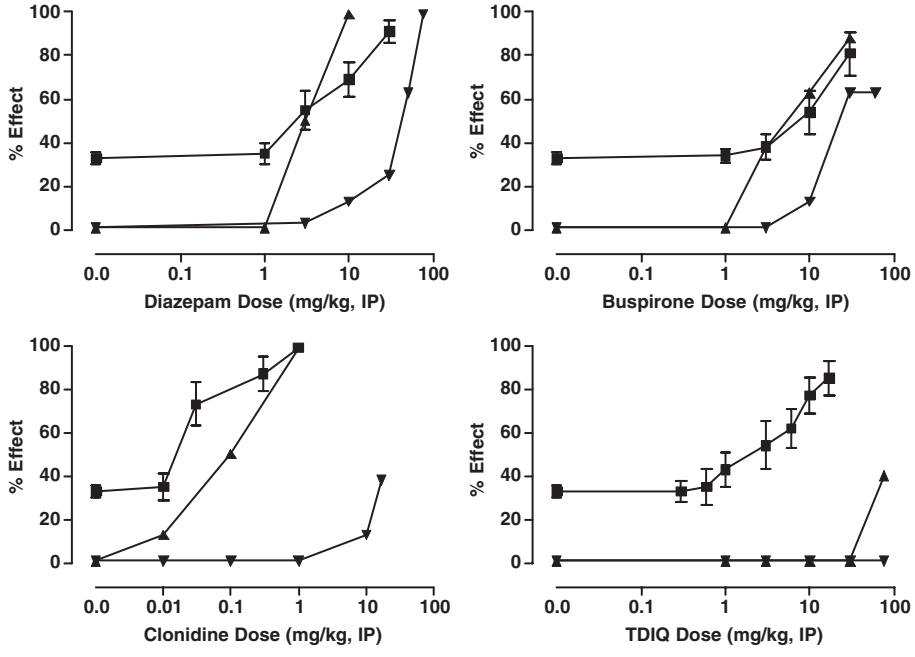

Fig. 2. Results of test agents on anxiolytic-like (i.e. percent increase of marbles that remained uncovered; ■—■), rotarod (i.e. percent of mice disrupted; ▲—▲), and inclined screen (i.e. percent of mice impaired; ▼—▼) activities in mice that were housed alone (i.e.1/cage) prior to testing. See Fig. 1 for further details.

Table 2
Comparison of test agents in mice that were housed alone on marble-burying (MB), rotarod (RR), and inclined screen (IS) tests; ns indicates no statistical difference ($p > 0.05$) between the dose–response functions for the activity ratio

Test agent	Marble-burying (MB)	Rotarod (RR)	Inclined screen (IS)	Ratio RR/MB	Ratio IS/MB
Diazepam	2.4 (0.8–7.1)	3.3 (1.95–5.49)	34.4 (19.5–61–2)	1.4^{ns}	14.4*
Buspirone	4.6 (1.5–14.3)	7.4 (3.60–15.2)	63% at 60 mg/kg	1.6^{ns}	CND > 31
Clonidine	0.016 (0.004–0.071)	0.06 (0.02–0.27)	38% at 17 mg/kg	3.0^{ns}	CND > 850
TDIQ	1.6 (0.5–4.4)	38% at 75 mg/kg	0% at 75 mg/kg	CND > 45	CND > 45

See Table 1 for further details concerning data entries, dose–response statistical evaluations, and ratio comparisons.

just 38% of mice exhibited incoordination at 75 mg/kg of TDIQ. It was noted (personal observation), however, that some (~60%) of the mice appeared to exhibit some signs of "sympathetic" activity (e.g., tremor, tachycardia) at 75 mg/kg of TDIQ, but these effects did not appear to influence their performance in the RR and IS tests.

3.2.3. Inclined screen (IS)

Diazepam (ED_{50} = 34.5 mg/kg) produced a dose-related impairment in this assay (Fig. 2 and Table 2). Buspirone and clonidine did not produce complete dose–response functions: maximal percent impairments of 63% and 38% of mice at 60 mg/kg of buspirone and 17 mg/kg of clonidine respectively (Fig. 2 and Table 2). The *z*-tests revealed that the response of the control group was statistically different ($p < 0.05$) from the following doses of diazepam (50 and 75 mg/kg) and buspirone (30 and 60 mg/kg). In comparison, TDIQ did not produce an effect in this procedure: 0% of mice were impaired at 75 mg/kg of TDIQ (Fig. 2 and Table 2).

3.2.4. Statistical comparisons between dose–response relationships

Statistical comparisons were performed between the dose–response relationships of the agents that inhibited marble-burying (MB) versus the dose–response functions that disrupted rotarod (RR) and inclined screen (IS) activities (Table 2). No statistical difference was noted between the dose–response relationship of diazepam, buspirone or clonidine that inhibited MB versus the dose–response function of each compound that disrupted RR activity. TDIQ produced an ED_{50} dose (1.6 mg/kg) for anxiolytic-like activity that is estimated to be over 45 times more potent than its highest tested dose that produced some impairment in the RR test (i.e. 38% of mice were impaired in the RR test at 75 mg/kg of TDIQ). In the MB test versus the IS procedure a statistical difference was noted in the dose–response relationship for diazepam ($F(1, 6) = 38.54$, $p < 0.05$). Buspirone, clonidine, and TDIQ did not produce complete dose–response functions in the IS and/or RR procedures (Fig. 2 and Table 2). Buspirone and clonidine produced ED_{50} doses (4.6 mg/kg and 0.016 mg/kg respectively) for anxiolytic-like activity that were estimated to be at least 13 times and greater than 850 fold more potent that their highest test dose that produced some impairment in the IS test (i.e. 63% and 38% of mice were impaired at 60 mg/kg of buspirone and 17 mg/kg of clonidine respectively). TDIQ produced an ED_{50} dose (1.6 mg/kg) for anxiolytic-like behavior that was estimated to be greater than 45 times more potent than its highest test dose that produced some, or no, impairment in the RR and IS tests (i.e. 38% and 0% of mice showed impairment in the tests at 75.0 mg/kg of TDIQ).

3.3. Between groups statistical comparisons

Table 3 details the statistical differences between the dose–response functions of the test agents in mice housed either alone or in groups on marble-burying (MB), rotarod (RR), and inclined screen (IS) tests. The evaluations revealed that the mice housed alone required statistically significant higher doses of diazepam (MB-alone versus MB-group, $F(1, 6) = 13.19$, $p < 0.05$), buspirone (MB-alone versus MB-group, $F(1, 4) = 40.80$, $p < 0.05$), and TDIQ (MB-alone versus MB-group, $F(1, 9) = 27.32$, $p < 0.05$) to exhibit anxiolytic-like behavior than mice housed in groups. The administration of clonidine did not result in a statistically significant difference between the two groups of mice. A comparison of RR activity indicated no statistical difference between the two groups of mice after the administration of diazepam, buspirone, or

Table 3
Comparison of test agents in mice that were housed either alone or in groups on marble-burying (MB), rotarod (RR), and inclined screen (IS) tests

Test agent	Marble-burying		MB ratio alone/group	Rotarod		RR ratio alone/group	Inclined screen		IS ratio alone/group
	Housed alone	Housed in groups		Housed alone	Housed in groups		Housed alone	Housed in groups	
Diazepam	2.4	0.26	9.2*	3.3	4.3	0.8^{ns}	34.5	34.4	1.0^{ns}
Buspirone	4.6	2.36	2.0*	7.4	7.8	1.0^{ns}	63% at 60 mg/kg	63% at 75 mg/kg	CND
Clonidine	0.016	0.018	0.9^{ns}	0.06	0.12	0.5^{ns}	38% at 17 mg/kg	57% at 30 mg/kg	CND
TDIQ	1.6	0.20	8.0*	38% at 75 mg/kg	13% at 100 mg/kg	CND	0% at 75 mg/kg	13% at 100 mg/kg	CND

Entry values are either the calculated ED_{50} dose (mg/kg) or the highest tested dose (with percent of effect) of an agent in the procedure. Where possible the ED_{50} dose ratios are presented for marble-burying, rotarod, and/or inclined screen activities in mice that were housed under each condition; for convenience, a ns (no statistical difference, $p > 0.05$) or an * (statistical difference, $p \leq 0.05$) is placed next to the ratio value to indicate statistical significance between the dose–response functions for a compound. If a test compound did not produce a complete dose–response curve in a procedure, then it is noted that the ratio and appropriate statistical comparison(s) could not be determined (CND).

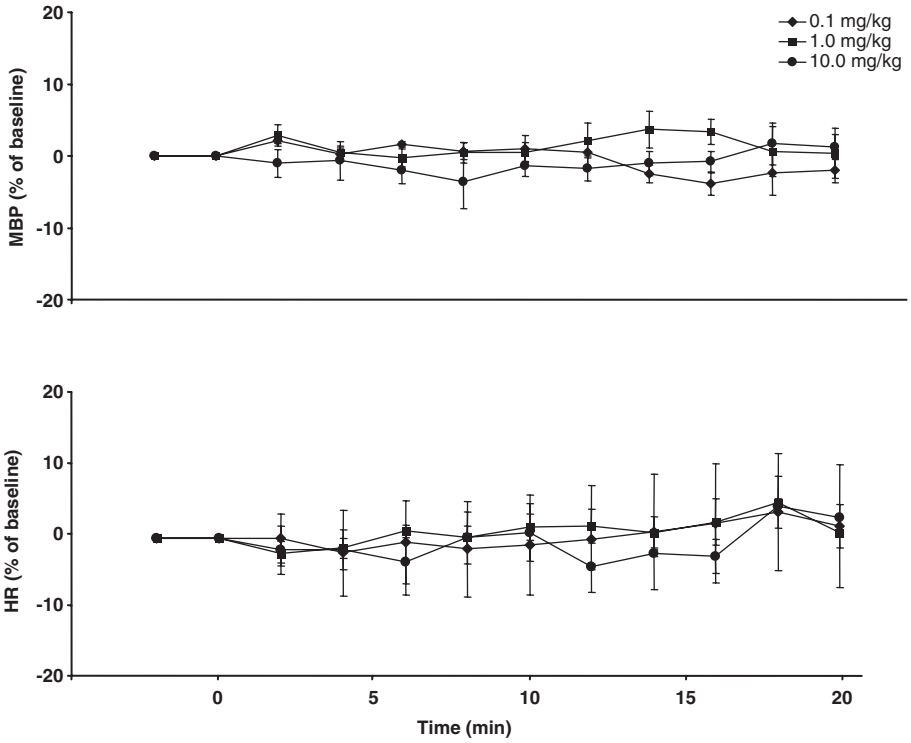

Fig. 3. Effects of TDIQ doses on blood pressure (top) and heart rate (bottom) in mice. Ordinate: mean (±S.E.M.; $n=10$ mice/dose) effect on blood pressure (mm Hg) and heart rate (beats/min) after the IV administration of various doses of TDIQ. Abscissa: time (at 2 min intervals) of measurement of the dependent variables.

clonidine. The administration of TDIQ did not produce a complete dose–response relationship in either group of mice and, therefore, an appropriate statistical comparison could not be determined (CND). Lastly, a comparison of IS performance indicated no statistical difference between the two groups of mice after the administration of diazepam. The administration of buspirone, clonidine, or TDIQ did not result in a complete dose–response relationship in either group of mice and, therefore, an appropriate statistical comparison CND.

3.4. Heart rate and blood pressure

Statistical analysis revealed that TDIQ, administered (IV) at 0.1, 1.0 and 10.0 mg/kg and measured every 2 min for 20 min, did not exert a significant effect on BP or HR (Fig. 3). It was observed that the maximum change in BP was 3.9 (i.e. ±1.6% of baseline) and HR was 4.8 (i.e. ±3.5% of baseline) following the administration of these doses of TDIQ (i.e. a 100 fold range of doses).

4. Discussion

Animals, such as mice and rats, oftentimes bury objects in the bedding material of their cage. One example of this type of behavior occurs when mice are placed in an environment, similar to their standard housing, which contains glass marbles as unfamiliar objects. Typically, the mice have been housed in a group (e.g. 5–30/cage), but are placed in the test environment on an individual basis and proceed to bury, within 30 min, approximately 70% of the marbles (e.g., Broekkamp et al., 1986; Njung'e and Handley, 1991). Animals that are pretreated with an antianxiety agent, however, will bury significantly fewer marbles. The administration of diazepam or buspirone, for example, inhibits marble-burying behavior (i.e. more marbles remain uncovered) at doses that do not disrupt motor activity (e.g., Chaki et al., 2003). In the present study, the anxiolytic agents blocked marble-burying behavior of mice as reported previously. As shown in Fig. 1 and Table 1, diazepam produced a dose-related increase in the percent of marbles left uncovered ($ED_{50}=0.26$ mg/kg: 0.9 μmol/kg) that was statistically different from its dose–response disruption of rotarod activity ($ED_{50}=4.3$ mg/kg) and impairment of inclined screen behavior ($ED_{50}=34.4$ mg/kg). Taken together, the results appear to parallel clinical observations that although benzodiazepines, such as diazepam, exhibit marked efficacy in the treatment of anxiety they also can produce side effects such as motor incoordination and significant sedation (e.g., Hollister et al., 1993). The azapirone buspirone produced a dose-related attenuation of marble-burying behavior ($ED_{50}=2.36$ mg/kg: 5.6 μmol/kg) that was statistically different from its dose-related disruption of rotarod behavior ($ED_{50}=7.8$ mg/kg) and, its ED_{50} dose in the marble-burying assay was >30 times more potent than the highest tested doses of buspirone that produced a maximal impairment in the inclined screen test; 63% of mice were impaired at 50 mg/kg or 75 mg/kg of buspirone (Fig. 1 and Table 1). The dose-related disruption of rotarod behavior in mice, after the acute administration of the drug, is not inconsistent with the clinical notation that patients may experience some degree of dizziness, incoordination, drowsiness, or fatigue upon initiation of buspirone therapy (e.g., Gelenberg, 1994). Moreover, the present result that the mice exhibited limited disruption (i.e. lack of a complete dose–response effect) of inclined screen activity may be consistent with

the clinical conclusion that buspirone is less likely to induce a marked CNS depressant effects as compared to other anxiolytic agents (e.g., Manfredi et al., 1991; Gelenberg, 1994).

Rodents that are housed alone have been proposed to be suitable subjects for an animal model of anxiety (e.g., Parker and Morinan, 1986; Wongwitdecha and Marsden, 1996). The present study evaluated mice that were housed singly. If those mice are more "anxious" than mice that are housed in a group, and the inhibition of marble-burying behavior is a suitable test to measure an anxiolytic-like effect, then it might be predicted that those mice would (a) bury more marbles under the saline vehicle (control) treatment, (b) perhaps perform poorly in the rotarod and/or inclined screen test, and (c) require higher doses of an anxiolytic agent to inhibit marble-burying behavior than mice housed in a group. The current results indicate that the control level of burying marbles was similar (i.e. ratio of approximately 70%/30% of marbles buried/uncovered, respectively) in both groups of mice (Figs. 1 and 2). The lack of a difference in results between control groups, however, could be the result of a "flooring" effect on marble-burying behavior. A search of the literature on marble-burying studies indicated that the ratio of 70%/30% of marbles buried/uncovered, respectively, appeared to be one with the greatest difference in the ratio (i.e. a low baseline level). There was also no difference in performance between the two control groups of mice in the rotarod test and the inclined screen assays (Figs. 1 and 2). That is, none of the animals assigned to the control condition, in either group of animals, were impaired in those procedures. However, a much higher number of mice that were housed alone than mice that were housed in a group were required to be tested in order to meet the qualifying criterion for compound testing in the rotarod test (see Materials and methods and Results). Finally, Table 3 reveals that the anxiolytic-like dose–response functions of diazepam and buspirone were found to be statistically different (i.e. less potent) when administered to mice that were housed alone (ED_{50} values of 2.4 mg/kg and 4.6 mg/kg, respectively) as compared to mice that were housed in a group (ED_{50} values of 0.26 mg/kg and 2.36 mg/kg, respectively). Thus, mice that were housed alone required statistically significant higher doses of the antianxiety drugs to exhibit anxiolytic-like behavior (Table 3). In the rotarod and inclined screen tests, however, these anxiolytic compounds produced dose–response functions that are very similar in both groups of mice (Figs. 1 and 2, Table 3).

Fig. 2 and Table 2 show more specifically the results obtained with the anxiolytic agents in the mice that were housed alone. Diazepam produced a dose-related increase in the number of marbles left uncovered (ED_{50}=2.4 mg/kg; 8.4 μmol/kg) that was not statistically different from its dose-related disruption of rotarod activity (ED_{50}=3.3 mg/kg), but it was statistically different from its dose-related impairment of inclined screen behavior (ED_{50}=34.5 mg/kg). Buspirone also produced a dose-related decrease in marble-burying activity (ED_{50}=4.6 mg/kg; 10.9 μmol/kg) that was not statistically different from its dose-related disruption of rotarod behavior (ED_{50}=7.4 mg/kg), but its ED_{50} dose in the marble-burying test was 13 fold lower than the highest tested dose of buspirone that produced a maximal impairment in the inclined screen test: 63% of mice were impaired at 60 mg/kg of buspirone (Fig. 2 and Table 2). Taken together, the results with diazepam and buspirone demonstrate the occurrence of narrowed areas between the dose–response curves for anxiolytic-like activity and the dose–effect functions for potential side effects in the mice housed alone versus the mice housed in a group. That is, the dose–response functions of diazepam and buspirone were shifted to the right for marble-burying behavior in those animals housed alone (relative to those mice housed in groups) without concomitant shifts of the dose–response functions on rotarod and/or inclined screen activities (Figs. 1 and 2). Therefore, the results could be consistent with the idea that mice housed alone might be more "anxious" than mice housed in groups and, consequently, require a higher dose(s) of an anti-anxiety agent to produce anxiolytic-like behavior in the marble-burying assay. The fact that those mice required higher doses of the anxiolytic agents, however, appears to make them more susceptible (than the mice housed in groups) to the potential side effects of the agents because they did not require higher doses of those agents to disrupt their performance in the rotarod or inclined screen tests (Table 3). The human corollary could be that an individual who is diagnosed as having a relatively high degree of anxiety, as compared to another individual, might require more anxiolytic compound per day for clinical efficacy but might be considered more susceptible to the unwanted side effects of the medication.

It has been suggested that the central noradrenergic system may underlie some forms of human anxiety (e.g., Bremner et al., 1996). In particular, inappropriate or excessive release of norepinephrine has been associated with anxiogenic behavior (e.g., Bremner et al., 1996; Redmond and Huang, 1979). If some types of anxiety result, in part, from overactivity of brain noradrenergic systems, then it can be proposed that drugs that decrease noradrenergic function might have antianxiety effects. Clonidine, for example, has been shown to inhibit norepinephrine activity by stimulating α_2-noradrenergic autoreceptors, and reports have indicated that it may attenuate several types of anxiety (e.g., Carnwath and Hardman, 1998; Hoehn-Saric et al., 1981; Nilsson et al., 1985). In the present study, clonidine produced dose-related reductions of marble-burying behavior in mice housed either in a group or alone (ED_{50} doses=0.018 mg/kg (0.1 μmol/kg) and 0.016 mg/kg (0.1 μmol/kg), respectively), but these dose–response functions are almost identical and not statistically different from each other (Table 3). As shown in Figs. 1 and 2 (and Tables 1 and 2) the latter dose–response curves are close to the dose-related disruptions of coordinated movement in the rotarod test (ED_{50}=0.12 mg/kg and 0.06 mg/kg, respectively); these results are consistent with an earlier report of the effects of clonidine in a marble-burying assay and motor tests (Millan et al., 2000a,b). A greater separation of effects was observed, however, between the dose–response effect (and ED_{50} doses) of clonidine in the marble-burying assay and the highest tested doses of clonidine that produced maximal impairments in the inclined screen test: 57% of mice housed in a group were impaired at 30 mg/kg of clonidine and 38% of mice housed alone were impaired at 17 mg/kg of clonidine. Unfortunately, clonidine does not generate much clinical interest because of its presumed α_2-noradrenergically-mediated side effects (e.g., hypotension, sedation, dry mouth). However, clonidine also interacts

with transmitter systems such as α_1-adrenergic, imidazoline, and serotonin $5HT_{1A}$ receptors that can produce, or contribute to, such side effects (e.g., Gavras et al., 2001; Guyenet, 1997; Kolassa et al., 1989; Reis and Piletz, 1997) and, therefore, may not be the most suitable pharmacological probe of an α_2-noradrenergic receptor-mediated anxiolytic effect. In fact, the precise role of the noradrenergic system in mediating its pharmacological activities has likely been obscured by the relative non-selectivity of traditional noradrenergic agents.

In general, anxiolytic agents may produce a wide range of adverse effects, the most common being interference with coordinated movement(s) and over-sedation. Therefore, the demonstration of anxiolytic-like action by novel agents should not be compromised by sedative-like actions that can be demonstrated initially in preclinical animal models. Clearly, it would be advantageous for a purported new anxiolytic agent to exhibit a markedly reduced potential for such activities. In the present study, the putative α_2-noradrenergic agent TDIQ displayed a wide separation between doses that produced anxiolytic-like effects and doses that produced some, if any, degree of impairments in the rotarod, inclined screen, and HR/BP tests. Figs. 1 and 2 (and Tables 1 and 2) show that TDIQ produced dose-related increases in the percent of marbles that remained uncovered by mice housed either in a group (ED_{50}=0.20 mg/kg: 0.9 μmol/kg) or alone (ED_{50}=1.6 mg/kg: 7.5 μmol/kg); the latter dose–response curve was determined to be statistically different (i.e. less potent) from the former dose–response relationship (Table 3). Interestingly, the ED_{50} doses for TDIQ are quite comparable to the ED_{50} doses for diazepam. Unlike diazepam, however, the ED_{50} dose of TDIQ for anxiolytic-like activity, in the mice housed in groups, can be estimated to be at least 500 fold lower than the highest dose tested that produced some impairment in the rotarod and inclined screen tests (i.e. only 13% of mice were impaired in each test at 100 mg/kg of TDIQ). Similarly, the ED_{50} dose of TDIQ for anxiolytic-like behavior, in mice housed alone (Table 2), can be estimated to be >45 times less than the highest dose tested that produced some, or no, disruption in the rotarod and inclined screen assays (i.e. 38% and 0% of mice were impaired in the rotarod and inclined screen tests, respectively, at 75 mg/kg of TDIQ). Finally, a very interesting, and quite surprising, result of TDIQ was that it did not produce a marked effect on HR/BP in mice. Mice were administered intravenous doses of 0.1, 1.0 and 10 mg/kg of TDIQ prior to measurements of HR/BP that were recorded every 2 min for 20 min. As shown in Fig. 3 the administration of these doses of TDIQ had a negligible effect (i.e. dose effects within ±<5% of baseline values) on these important indicators of cardiovascular function. Thus, when the present results are taken together with the results of an earlier report (Malmusi et al., 1996), it can be concluded that TDIQ appears to exhibit a dose-dependent and wide dissociation between anxiolytic-like activity and potential impairments in locomotor, rotarod, inclined screen, and HR/BP tests. Moreover, it can be speculated that the anxiolytic-like effect of TDIQ might be produced by an attenuation of norepinephrine activity that occurred, perhaps, by an agonist effect at presynaptic autoreceptors or an antagonist effect at postsynaptic receptors. Further studies will be required, however, to characterize more fully the exact mechanism of action of TDIQ. Nevertheless, one explanation of the present results could be that the placement of a mouse into a familiar environment that contains unfamiliar objects (marbles) results in an efflux of norepinephrine, stimulation of postsynaptic α_2-noradrenergic receptors, and consequent behavioral excitation; the response of the rodent is to bury the marbles. The latter activity can be attenuated, however, in animals that are pretreated with TDIQ. The excessive or inappropriate release of norepinephrine via α_2-noradrenergic neurons has been speculated to underlie some types of anxiety (e.g., Bremner et al., 1996). In those situations, TDIQ could potentially influence the activity of α_2-noradrenergic neurons and lead to a restoration of balance between excitatory and inhibitory processes in α_2-noradrenergic areas of the brain. Thus, TDIQ may be able to restore α_2-noradrenergic "homeostasis" by an improvement of inhibitory synaptic control and, consequently, produce an anxiolytic effect.

Historically, α_2-noradrenergic receptors have been thought to exert a crucial role in the genesis of sedation and adverse cardiovascular reactions (e.g., Gavras et al., 2001). The discovery and development of a putative α_2-noradrenergic agent that seems mostly devoid of the aforementioned side effects could lead to a clearer characterization of the functional role of the α_2-noradrenergic receptor and might result in an improved and/or advanced clinical medication. To date, TDIQ appears to be a very selective compound for α_2-noradrenergic receptors, does not seem to produce marked motor impairment, and has little effect on HR/BP in mice (Glennon et al., 2002; Malmusi et al., 1996; Young and Glennon, 2002; present data). As such, the present results may have significant clinical implications.

It has been suggested that the noradrenergic system may exert a critical role in the symptoms of anxiety that occur in a variety of human disorders such as PTSD, panic, OCD, insomnia that is linked to anxiety, "sundowning" in patients with dementia, behavioral problems associated with PMS, and anxiety that arises from drug abuse withdrawal syndromes. Although neurochemical studies of those disorders have led to various explanations for their occurrence, an increased reactivity of α_2-noradrenergic neurons is linked, at least in part, to some of the anxiety symptoms that appear in these conditions. For example, PTSD occurs in some individuals following the experience of a severely distressing event such as a war experience(s), natural disaster, violent crime, or rape and is characterized by symptoms of hyperarousal, hypervigilance, intrusive re-experiencing, sleep disturbance, and difficulty with concentration (e.g., Bremner et al., 1996). Panic disorder is characterized by the occurrence of a sudden, intense, and distinct wave of symptoms such as loss of control, fear of dying, racing heart rate, shortness of breath, and dizziness (Heninger et al., 1988). OCD symptoms are recurrent obsessions or compulsions that are severe enough to be time-consuming or cause marked distress or significant impairment (APA, 2000). Insomnia that is associated with anxiety may involve an inappropriate or excessive degree of alertness that is closely associated with neuronal firing activity of α_2-noradrenergic neurons in the locus coeruleus (e.g., Berridge and Waterhouse, 2003). Patients who are cognitively impaired may exhibit a "sundowning" syndrome that results in agitated, anxious, or confused behavior(s) toward the end of the day. It is noteworthy, although not conclusive evidence, that patients with Alzheimer's disease, particularly those in more advanced stages of the disease,

have increased levels of norepinephrine and a greater density of noradrenergic receptors in the brain on autopsy (e.g., Hoogendijk et al., 1995). Some women with PMS will experience significant mood disruptions that manifest, for example, as irritability and anxiety. An α_2-noradrenergic agonist effect by clonidine has been reported to alleviate those symptoms (e.g., Nilsson et al., 1985). Lastly, an individual's withdrawal from drugs of abuse such as alcohol, opiates, and cocaine may produce anxiety symptoms that are linked to hyperactivity of the α_2-noradrenergic system (e.g., Carnwath and Hardman, 1998; McDougle et al., 1994). If overactivity of the α_2-noradrenergic system plays a significant, or contributory, role in the above conditions, then it might be speculated that the administration of TDIQ could function as a "surge limiter or protector" that dampens the excessive or inappropriate activity of the system. Consequently, the neurochemical action of TDIQ might temporarily reduce the synaptic concentration of norepinephrine and provide an anxiolytic effect.

In summary, the present study compared the anxiolytic-like activity and the potential side effects of the α_2-noradrenergic agent TDIQ to those of the reference compounds diazepam, buspirone, and clonidine in mice. The results indicated that, in comparison to those agents, TDIQ displayed the widest separation between doses that produced anxiolytic-like activity and doses that disrupted coordinated movement and/or general motor activities. In fact, TDIQ did not produce dose-related impairments in the latter side effect tests. In addition, the administration of IV doses of TDIQ to mice did not markedly alter their heart rate or blood pressure. As such, the data suggest that TDIQ could (a) exhibit a significantly favorable therapeutic effect to side effect ratio, (b) be used as a pharmacological probe to characterize more clearly the actions of α_2-noradrenergic receptors and (c) serve as a possible chemical template in the discovery and development of additional agents that might be selective for α_2-noradrenergic receptors. Overall, it is reasonable to conclude from the available animal data that TDIQ may represent a prototype agent for a possible new generation of antianxiety agents.

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