ELSEVIER

Pharmacology, Biochemistry and Behavior 73 (2002) 537–544

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Behavioral responses to stress following central and peripheral injection of the 5-HT_2 agonist DOI

Mike F. Hawkins*, Sarah M. Uzelac, Alan A. Baumeister, John K. Hearn, John I. Broussard, Thomas S. Guillot

Department of Psychology, Louisiana State University, Baton Rouge, LA 70803, USA

Received 7 February 2002; received in revised form 20 March 2002; accepted 29 March 2002

Abstract

Evidence suggests that serotonin (5-HT) systems are involved in the regulation of an organism's response to stress. Experiments were conducted to evaluate the possibility that central (20, 100, or 200 μg icv), peripheral (0.1, 0.5, or 1.0 mg/kg sc), or combined central (200 μg) plus peripheral (0.1 mg/kg) injections of the selective 5-HT_2 agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) would alter behavioral responses to stress in rats. Animals were evaluated during tail pinch stress, in an open field, and on a rotarod task. Across the three modes of administration (icv, sc, icv + sc), DOI resulted in a dose-related decrease in five of seven classes of behaviors observed during tail pinch. This reduction was most pronounced following subcutaneous injections, but occurred following intracerebroventricular and combined subcutaneous and intracerebroventricular injections as well. An additive effect of combined intracerebroventricular and subcutaneous administration was suggested by the fact that doses which were ineffective when given singly by these two routes resulted in a reduction in stress-evoked behavior when given together. Reduced responding seemed not to be attributable to general motoric impairment as DOI did not affect locomotion, grooming, or rotarod performance. The results suggest that activation of 5-HT_2 receptors produces an anxiolytic effect in rats subjected to acute tail pinch stress.

Keywords: Stress; Tail pinch; DOI; Serotonin

1. Introduction

Serotonin (5-HT) systems are known to be involved in a variety of behavioral and physiological processes related to an organism's response to stress. A consensus has not been reached, however, regarding the precise nature of this involvement, as some findings suggest an anxiogenic action of 5-HT while others indicate an anxiolytic profile (see Zangrossi et al., 2001 for a review). The identification of multiple 5-HT receptor subtypes and the advent of increasingly selective ligands have led to a wealth of information regarding the roles these receptors may play. Evidence implicates 5-HT_2 receptors in the regulation of the stress response but, as with 5-HT generally, whether stress activates or inhibits 5-HT_2 systems is a matter of current discussion.

Among the findings which indicate that stress is associated with decreased 5-HT_2 activity are reports that elevated plasma ACTH levels (Kuroda et al., 1992), or stressors such as, restraint, inescapable electric shock, or chronic social stress, increase the number of 5-HT_2 binding sites in the cortex (McKittrick et al., 1995; Torda et al., 1988) and increase the sensitivity of animals to 5-HT_2 agonists such as (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) (Chaouloff et al., 1994; Gorzalka et al., 1998; Nankai et al., 1995). These effects are consistent with the notion that both chronic and acute stressors decrease activity in 5-HT_2 systems, which results in compensatory receptor up-regulation and increased receptor sensitivity.

Similarly, several of the reported actions of DOI suggest a sympathoinhibitory and anxiolytic profile. Systemic administration of DOI produces dose-dependent reductions in defensive burying (Njung'e and Handley, 1991) and schedule-induced polydipsia (Lu et al., 1992), which are reversed by selective 5-HT_2 antagonists. Central injection of DOI has been reported to decrease plasma corticosterone (Welch and Saphier, 1994), to decrease activity in the renal sympathetic nerve, to decrease renal blood flow, and to produce bradycardia (Alper, 1990; Anderson et al., 1992).

* Corresponding author. Tel.: +1-225-578-4660; fax: +1-225-578-4672.
E-mail address: pshawk@lsu.edu (M.F. Hawkins).

0091-3057/02/$ – see front matter
PII: S0091-3057(02)00822-5

Conversely, several effects of DOI are consistent with a sympathoexcitatory and anxiogenic action. Central injections have been shown to activate the hypothalamic–pituitary–adrenal axis (Raghavendra and Kulkarni, 2000), to increase plasma corticosterone (Welch and Saphier, 1994), and to produce hypertension and tachycardia (Anderson et al., 1992; Bell et al., 1999). Peripheral injections are reported to increase sympathetic nerve activity (McCall and Clement, 1994), to elevate plasma levels of corticosterone and epinephrine (Chaouloff, 1993), and to inhibit sexual behavior (Gorzalka et al., 1998).

The purpose of the experiments reported below was to investigate the possibility that activation of 5-HT_2 systems by DOI would alter behavioral responding to acute stress. Animals were evaluated using two stress paradigms: tail pinch stress and open-field responding. Additionally, performance on a rotarod task was examined as a control for general motor impairment produced by drug administration. Because DOI has been shown to alter physiological and behavioral variables following both central and peripheral routes of administration, behavioral measures were obtained in separate groups of animals that received central, peripheral, or central plus peripheral injections.

2. Method

2.1. Subjects

Male Sprague–Dawley rats were used. Animals were obtained from the Division of Laboratory Animal Medicine, School of Veterinary Medicine, Louisiana State University. They were housed individually with food and water available at all times. The vivarium was maintained at a temperature of 22 °C, and lighting was cycled on a 12-h photoperiod (on 07:00 h). Behavioral testing occurred during the animals' light phase, commencing at 09:00 h.

2.2. Drug

DOI was obtained from Sigma. It was dissolved in sterile water and dispensed into aliquots, which were lyophilized and stored frozen until needed. The drug was reconstituted in sterile 0.9% saline just prior to injection.

2.3. Surgery

Animals that received central injections underwent stereotaxic surgery for implantation of bilateral intracerebroventricular cannulae when they reached a body weight of 280–320 g. The animals were anesthetized with ketamine HCl (90 mg/kg im) and xylazine (5 mg/kg im), and the skull surface was irrigated with 0.25–0.5 ml of a lidocaine HCl (2%)/bupivacaine HCl (0.5%) solution to reduce postoperative pain. Stainless-steel guide cannulae were implanted 0.0 mm anterior to bregma, ±1.6 mm lateral from the midline, and −3.0 mm ventral to dura (Pellegrino et al., 1979). These were affixed with dental acrylic to stainless-steel screws placed in the skull surface. Animals were given 5 days of postoperative recovery before experimental testing began. This surgical protocol was approved by the Louisiana State University Institutional Animal Care and Use Committee (protocol no. 99-115).

2.4. Injection procedure

Three experiments were conducted to evaluate possible effects of DOI on stress responding following central and peripheral administration. In Experiment 1, animals received subcutaneous injections of saline vehicle ($n=15$) or DOI in doses of 0.1 ($n=9$), 0.5 ($n=12$), or 1.0 ($n=12$) mg/kg. In Experiment 2, intracerebroventricular injections of saline ($n=14$) or 20 μg ($n=13$), 100 μg ($n=14$), or 200 μg ($n=14$) of DOI were used. In Experiment 3, both subcutaneous and intracerebroventricular injections were given. One group ($n=12$) received intracerebroventricular and subcutaneous injections of saline and a second group ($n=11$) was injected with 200 μg of DOI centrally and 0.1 mg/kg of DOI peripherally. Doses employed were determined based upon a review of the literature and pilot experiments conducted in our laboratory.

2.4.1. Peripheral injections

Subcutaneous injections were administered at the nape of the neck in a volume of 1 ml/kg. After injection, animals were replaced in their home cages for 30 min prior to tail pinch stress.

2.4.2. Central injections

Animals were removed from their home cages and were restrained by hand. Using a Sage infusion pump, saline or DOI was administered to the two ventricles simultaneously at a rate of 5.0 μl/min through sterilized injectors, which extended 1.0 mm below the ventral tip of the guide cannulae. A volume of 10 μl was delivered to each side over a 2-min period. The doses described above refer to the total amount of DOI an animal received. The animals were held for one additional minute after the injection to allow for the diffusion of drug from the injection site. Following central injections, animals were immediately exposed to the tail pinch stress.

2.4.3. Double injection procedure

Animals that received both peripheral and central injections received the subcutaneous injection first (saline or 0.1 mg/kg DOI, depending upon group assignment) and were returned to their home cages. After 25 min had elapsed, the animals were again removed from their home cages and were given intracerebroventricular injections (saline or 200 μg DOI). Procedures employed for the central and peripheral injections were as described above. Behavioral testing commenced immediately following the completion of the central injection.

2.5. Behavioral testing

The behavior of each animal was evaluated under three conditions: during tail pinch stress, in an open field, and on a rotarod apparatus. Behavioral data were collected by observers who were blind regarding drug condition and who had been trained to a criterion of 90% accuracy on each dependent variable relative to an "expert" observer.

2.5.1. Tail pinch condition

Following central and/or peripheral injection the animal was placed in a suspended stainless-steel cage, which contained a preweighed amount of laboratory chow. The tail of the animal was passed through the grid floor of the cage and a pair of modified hemostatic forceps, padded with latex tubing, was applied to the tail at a predetermined point along its length corresponding to a diameter of 4.3 mm. The pair of forceps was left in place for 4 min, during which data on the following seven variables were recorded:

1. *Oral behavior involving food* was recorded as the duration of behaviors such as licking, biting, chewing, eating, or gnawing, which were directed at food.
2. *Eating*. The amount of food an animal ate was defined as the difference in weight of lab chow placed in the cage prior to tail pinch and the amount remaining in and under the cage after tail pinch.
3. *Gnawing*. The amount of food gnawed was determined by weighing particles of lab chow which fell through the wire floor of the testing cage during tail pinch.
4. *Grooming* was measured as the amount of time an animal licked itself or combed its fur with its paws.
5. *Oral behavior not involving food or self* was measured as the duration of oral behaviors such as teeth chattering, vacuous chewing, and licking or biting of the cage.
6. *Vocalization* was recorded as the number of instances.
7. *Defecation* was recorded as the number of fecal boli.

Measurements of duration above were taken to the nearest 0.01 s, and measurements of weight were recorded to the nearest 0.01 g.

2.5.2. Open-field condition

Ten minutes following the completion of tail pinch testing, animals were placed in an open-field apparatus ($61 \times 61 \times 61$ cm), the floor of which was marked into quadrants of equal size. Behavioral observations of the following six variables were conducted for 4 min:

1. *Line crosses*: the number of lines that an animal crossed with both forepaws (Angulo et al., 1991; Lemoine et al., 1990).
2. *Rearing*: the number of times an animal lifted both forepaws from the floor.
3. *Freezing*: the amount of time (to the nearest 0.01 s) an animal remained motionless.
4. *Wet dog shakes*: the number of times an animal arched its back and shook its head, shoulders, and torso.
5. *Head shakes*: the number of times an animal shook its head only, without accompanying movement of the shoulders and torso.
6. *Defecation*: the number fecal boli.
7. *Flattened body posture*: the presence, for more than 10 consecutive seconds, of a body posture characterized by absence of an arched torso when the animal was stationary or during locomotion.

2.5.3. Rotarod test

Animals were trained on a rotarod apparatus 24 h following open-field testing. The apparatus consisted of a stainless-steel drum (36×7 cm diameter), covered with stainless-steel mesh, which was calibrated to rotate at 10 rpm. Training involved repeatedly placing an animal on the moving drum until it could remain on the apparatus for 30 consecutive seconds. Animals that did not meet this criterion were not used in subsequent tests for motor impairment. Twenty-four hours following rotarod training, animals were injected with saline or the dosage of DOI received during stress testing. Those that received intracerebroventricular injections were immediately placed on the apparatus for 30 s, or until they fell from the rotarod. Thirty minutes was allowed to elapse before the same procedure was performed on those animals that received subcutaneous injections. Latency to loss of balance, if it occurred, was recorded to the nearest 0.1 s.

2.6. Histology

At the conclusion of testing animals that received intracerebroventricular injections were sacrificed with an overdose of anesthetic and were perfused transcardially with 30 ml of physiologic saline followed with 30 ml of phosphate-buffered formalin. Brains were extracted, stored in formalin, and 80-μm sections were taken through the site of implantation using a freezing microtome. These were examined under a microscope. Only data from animals with confirmed bilateral placement of cannulae in the lateral ventricles were used in subsequent analysis.

2.7. Statistical analyses

Observations were taken on a total of 15 dependent variables (described above). Data regarding the presence or absence of flattened body posture were subjected to chi-square analysis. Rotarod data were analyzed with a univariate analysis of variance (ANOVA). The remaining 13 dependent measures were subjected to multivariate analysis of variance (MANOVA). A separate MANOVA was conducted for each of the three modes of drug administration (sc, icv, and sc + icv). A significant MANOVA result was followed by univariate ANOVAs for all dependent varia-

bles. Significant ANOVA results were further evaluated for dose–response effects using the Least Significant Difference test. Alpha was set at $P=.05$ for all comparisons.

3. Results

3.1. Experiment 1

MANOVA revealed a significant effect of peripherally administered DOI on behavioral responding [$F(39,102)=3.25$; $P=.00$].

3.1.1. Tail pinch condition

Data obtained in the tail pinch condition are displayed in Fig. 1. During tail pinch DOI significantly altered responding in four of the seven behavioral categories: oral behavior directed at food [$F(3,44)=3.04$; $P=.04$], gnawing [$F(3,44)=2.97$; $P=.04$], vocalization [$F(3,44)=3.81$; $P=.02$], and oral behavior not involving food or self [$F(3,44)=5.19$; $P<.01$]. Post hoc analysis of these variables revealed the following.

Oral behavior directed at food was not affected by the lowest dose (0.1 mg/kg) of DOI. The middle dose (0.5 mg/kg), however, significantly reduced responding relative to both saline ($P=.01$) and the low dose ($P=.04$). The high dose (1.0 mg/kg) produced an effect that was statistically indistinguishable from the middle dose ($P=.52$), but it only approached significance relative to saline ($P=.06$).

Gnawing following the low dose and saline was not different. Significantly less gnawing was observed following the middle ($P=.01$) and high ($P=.02$) doses relative to the low dose, but not relative to saline.

As with gnawing, the low dose did not alter vocalization, while the middle ($P<.01$) and high ($P=.01$) doses significantly decreased vocalization relative to the low dose.

Oral behavior not involving food or self was not affected by the lowest dose but was reduced significantly following the two higher doses. After the middle dose, duration of oral behavior was significantly lower than in either the saline

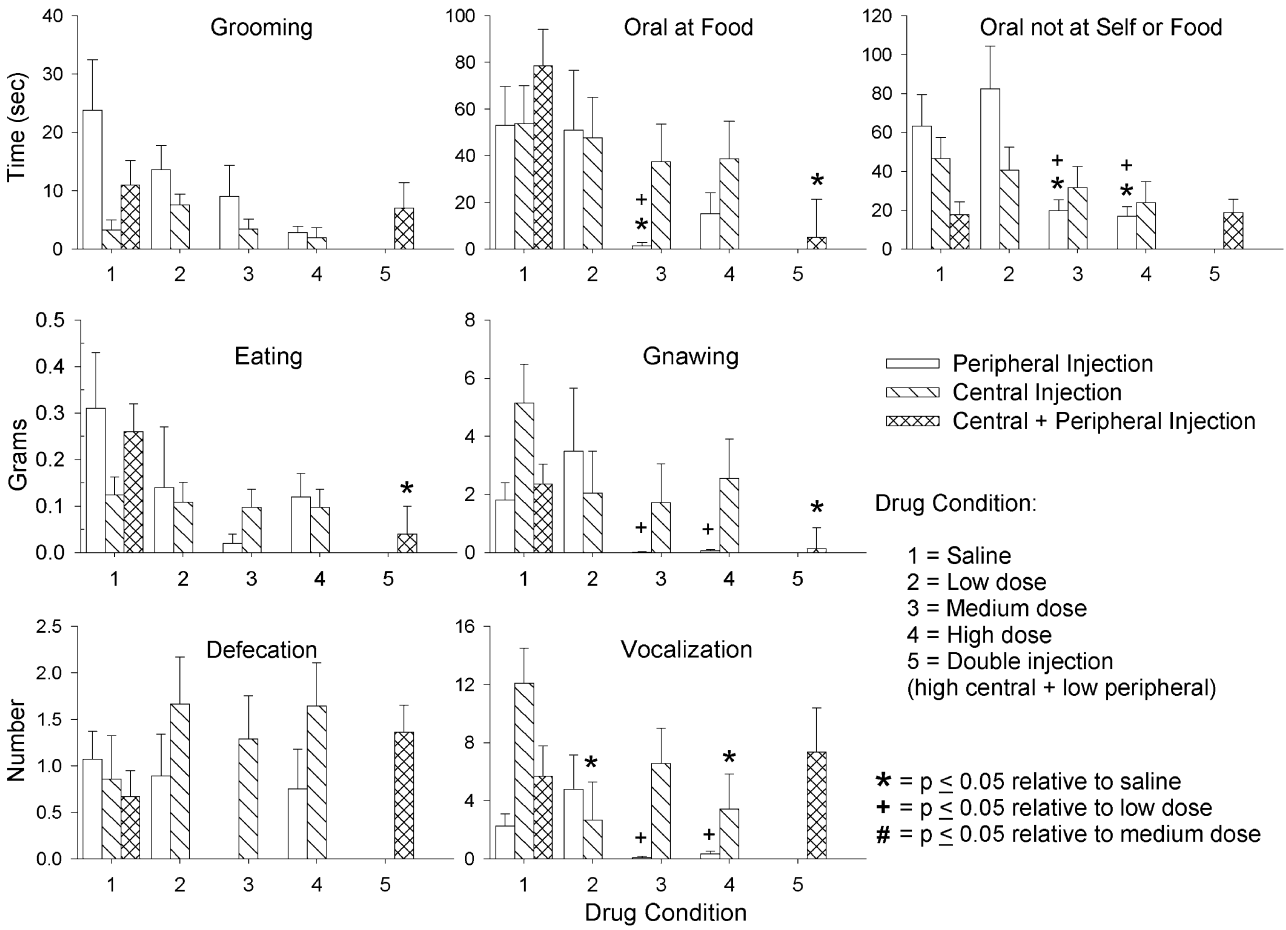

Fig. 1. Mean (±S.E.) behavioral responding to tail pinch stress following peripheral, central, or combined central and peripheral administration of DOI. Peripheral doses were 0.1 mg/kg (low dose), 0.5 mg/kg (medium dose), and 1.0 mg/kg (high dose). Central doses were 20 μg (low), 100 μg (medium), or 200 μg (high).

(P=.02) or the low dose (P<.01) condition. The same pattern of results relative to saline (P=.01) and the low dose (P<.01) was seen in the high-dose group. The effects of the middle and high doses did not differ from one another.

3.1.2. Open-field condition

Open-field data are displayed in Fig. 2. Significant effects of DOI were observed in six of the seven open-field variables: rearing [$F(3,44)=26.91$; $P<.01$], head shakes [$F(3,44)=9.26$; $P<.01$], wet dog shakes [$F(3,44)=7.21$; $P<.01$], freezing [$F(3,44)=3.54$; $P=.02$], defecation [$F(3,44)=3.99$; $P=.01$], and flattened body posture [$\chi^2(3)=34.29$; $P<.01$] (data not shown). The number of lines crossed was not significantly affected by DOI. Post hoc analysis demonstrated that rearing and defecation were significantly reduced following DOI while head shakes, wet dog shakes, freezing, and flat body posture were significantly increased.

All three doses of DOI significantly reduced the number of times animals reared relative to the saline group (P<.01 for each comparison). This reduction was greater for the middle dose than for the low dose (P=.04).

Defecation was also reduced by the low (P=.02), middle (P=.01), and high (P=.01) doses of DOI. These effects were statistically equivalent.

An increase in the number of head shakes was seen following both the middle and the high doses of DOI, and wet dog shakes were increased by the high dose (P<.01 for all comparisons).

The duration of freezing was increased by the middle (P=.04) and high (P=.03) doses. These effects did not differ from one another.

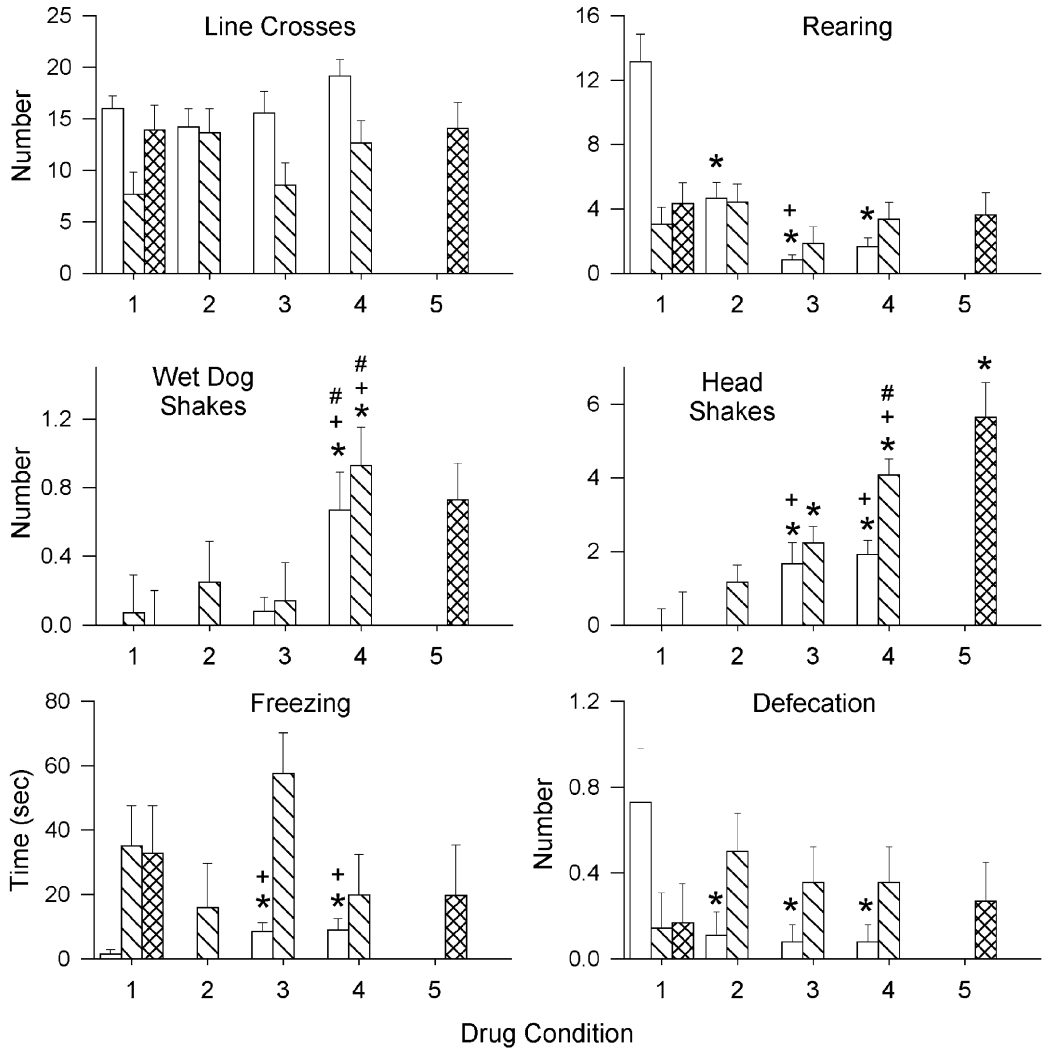

Fig. 2. Mean (±S.E.) behavioral responding in an open field following peripheral, central, or combined central and peripheral administration of DOI. Doses and legend are as in Fig. 1.

Flattened body posture was not observed in any animal that received either saline ($n=15$) or the low dose of DOI ($n=9$). In the middle- and the high-dose groups ($n=12$ each), however, this posture was observed in 83% of the animals (data not shown).

3.1.3. Rotarod test

Rotarod performance was not affected by DOI (data not shown). Animals in the saline condition ($n=14$) remained on the rotarod for an average of 26.6 s. Following the low ($n=9$), middle ($n=11$), and high ($n=11$) doses of DOI latencies to loss of balance were 28.1, 27.7, and 28.1 s, respectively.

3.2. Experiment 2

A significant effect of centrally administered DOI was determined by MANOVA [$F(39,120)=1.84$; $P=.01$]. This effect was restricted to vocalization [$F(3,50)=3.00$; $P=.04$] in the tail pinch condition (see Fig. 1) and to head shakes [$F(3,50)=15.94$; $P<.01$] and wet dog shakes [$F(3,50)=3.20$; $P=.03$] in the open-field condition (see Fig. 2). Vocalization was reduced by the low (20 μg) and high (200 μg) doses of DOI ($P=.01$ and $P=.02$, respectively). These reductions did not differ from the effect of the middle dose (100 μg) and did not differ from one another. The number of head shakes was increased significantly by the middle ($P<.01$) and high doses ($P<.01$). The effect of the high dose differed from the low dose and from the middle dose ($P<.01$). The number of wet dog shakes was increased by the high dose only ($P<.01$).

Flattened body posture was also significantly affected by dose of DOI [$\chi^2(3)=13.35$; $P<.01$] (data not shown). While no animal in the saline group ($n=14$) displayed this posture, it was observed in 8% of the low-dose group ($n=13$), 50% of the middle-dose group ($n=14$), and 42% of the high-dose group ($n=12$).

As with peripheral injections of DOI, central administration did not alter rotarod performance (data not shown). Latencies to loss of balance for the saline group ($n=11$) and low ($n=11$)-, middle ($n=9$)-, and high ($n=12$)-dose groups were 26.0, 26.8, 27.0, and 22.7 s, respectively.

3.3. Experiment 3

MANOVA revealed a significant effect of DOI on responding [$F(13,9)=3.60$; $P=.03$]. In the tail pinch condition (Fig. 1), this effect was evident as reductions in oral behavior directed at food [$F(1,21)=10.47$; $P<.01$], eating [$F(1,21)=6.53$; $P=.02$], and gnawing [$F(1,21)=4.91$; $P=.04$]. During open-field testing (Fig. 2), DOI significantly increased the number of head shakes [$F(1,21)=18.72$; $P<.01$] and wet dog shakes [$F(1,21)=6.26$; $P=.02$].

Rotarod performance was not significantly affected by combined central and peripheral injections of DOI. The mean latency for the saline group ($n=8$) was 26.8 s, and for the DOI group ($n=9$), it was 19.8 s.

4. Discussion

Across all behavioral variables and modes of administration, DOI resulted in a dose-dependent change in behavior consistent with a decreased responsiveness to tail pinch stress. Following peripheral injection, DOI decreased the amount of time animals engaged in oral behaviors directed at food. While this effect appeared to be attributable to a reduction in gnawing, DOI did not result in a statistically significant reduction in gnawing or eating relative to saline control. Significant reductions were also observed in the duration of oral behaviors such as teeth chattering, vacuous chewing, and licking or biting of the cage. Central injection of DOI resulted in a reduction in stress-evoked vocalization, while combined central and peripheral injection produced reductions in oral behavior directed at foods that were evident in measurements of both gnawing and eating. In no instance was behavior produced, which suggested an increased reaction to tail pinch stress.

The possibility that these reductions in responding are attributable to a nonspecific impairment of motoric response is made less plausible by the observation that DOI did not significantly affect grooming, locomotion (as measured by line crosses), or rotarod performance at any of the doses tested. While the lack of effect of DOI on locomotion reported here confirms previous work (Raghavendra and Kulkarni, 2000; Redrobe and Bourin, 1997), increases (Darmani et al., 1996; Granoff and Ashby, 1998) and decreases (Kaur and Ahlenius, 2000; Krebs-Thomson and Geyer, 1996) in locomotion have also been reported. Factors such as variations in circadian responsiveness to DOI (Nagayama and Lu, 1996) may play a role in these differences.

The pattern of results observed in the tail pinch test suggests that activation of 5-HT_2 systems centrally and/or peripherally results in an anxiolytic effect. Such a suggestion is congruent with other findings in the literature. In rats, peripheral and central injections of 5-HT_2 agonists (including DOI) have been shown to decrease ultrasonic vocalizations elicited by foot shock (Sanchez and Mork, 1999), decrease escape responding (Mora et al., 1997; Zangrossi et al., 2001), and decrease the aversiveness of electrical stimulation of the brain (Melo and Brandao, 1995; Nogueira and Graeff, 1995). Blockade of 5-HT_2 receptors, on the other hand, has been reported to increase ultrasonic stress vocalizations in rat pups (Olivier et al., 1998) and to increase subjective reports of anxiety in humans (Graeff et al., 2001; Silva et al., 2001). Nevertheless, the possibility that the decreases in behavioral responding observed in the present studies might reflect changes in variables such as nociceptive threshold to tail pinch, rather than an anxiolytic effect of DOI, warrants future consideration.

In the open field, peripherally administered DOI produced two effects that could be interpreted as anxiogenic actions: an increase in freezing behavior and a decrease in rearing. While the decrease in rearing reported here confirms a previous report (Kaur and Ahlenius, 2000), the

interpretation of the current findings is complicated by responding observed during the control conditions for these two variables. Following peripheral injection of saline, rearing was substantially elevated relative to the number of instances of rearing recorded following central and central plus peripheral saline injections. Additionally, the amount of rearing observed following peripheral injections of the three doses of DOI was comparable in magnitude to levels seen after central and central plus peripheral injections. Similarly, the duration of freezing behavior observed following peripheral saline administration was substantially less than was seen in the central or central plus peripheral control groups. Whether these differences in baseline responding are attributable to random variation or to differences inherent in the protocols (e.g., differences in animal handling required for central vs. peripheral injections) must await further investigation.

The most consistent behavioral responses observed in the open-field test were dose-related increases in head shakes and wet dog shakes evoked by DOI. However, these responses, as well as increases in freezing behavior observed in the open field, did not appear to act as significant competing responses in the tail pinch condition due to the relatively low frequency of their occurrence relative to session length. In the open field, a maximum of five head shakes and one wet dog shake were displayed on average. The maximum significant duration of freezing behavior was approximately 10 s. During tail pinch, significant reductions were observed in the duration of oral behaviors directed at food and oral behaviors not directed at self or food. These reductions occurred from a baseline rate of approximately 60 s each in control animals. That is, approximately 2 min of the 4-min testing session was devoted to these two classes of behavior. The remaining half of the testing period provided ample opportunity for the occurrence of even the maximum number of head shakes, wet dog shakes, and freezing behavior observed in the open field. Additionally, informal observations in the present studies corroborated a previous report that tail pinch stress decreases the frequency of occurrence of head shakes induced by DOI (Yamada et al., 1995).

That DOI affected wet dog shakes and head shakes following both central and peripheral routes of administration supports the suggestion of others that these behavioral responses are centrally mediated (Dey, 1994; Nankai et al., 1995). Whether the other behavioral effects of DOI observed in the open field and during tail pinch stress are attributable to a central mode of action remains unclear. The fact that 6 of the 10 behavioral variables that were found to be significantly affected by peripheral injection were not significantly altered by central injection argues against a central site of action. Furthermore, the lack of effect following central administration would seem not to be attributable to an insufficient concentration of DOI, as the 200-μg dose represents the maximum solubility of the drug in water (10 μg/μl). It is worth noting, however, that while stress-evoked oral behaviors were not significantly reduced by central DOI injections in the tail pinch condition, the data suggested a dose-related trend toward reduction. This was seen in the same three variables that were significantly reduced following peripheral injection: duration of oral behavior directed at food, gnawing, and oral behavior not directed at self or food. Additionally, the results of paired peripheral and central injections revealed that while neither the high dose given centrally nor the low dose given peripherally significantly altered food-related oral behavior when given alone, these two doses given together resulted in a significant decrease in eating, gnawing, and the duration of time that animals engaged in oral behavior directed at food. This additive effect of central and peripheral administration is consistent with the hypothesis that DOI acts centrally, as well as peripherally.

It has been reported that 5-HT ligands produce a more potent effect when injected into the cisterna magna than into the lateral ventricles (McCall and Clement, 1994). While it is possible that microinjections of DOI into the ventricular system of the lower brainstem might result in a different pattern of results than was observed here, a more fruitful line of inquiry might involve intraparenchymal sites. Graeff et al. have suggested that increased 5-HT activity in the ascending dorsal raphe pathway innervating the periaqueductal gray, the tectum of the midbrain, and the amygdala inhibits innate fear (Zangrossi et al., 2001). In this regard, microinjections of DOI and other 5-HT_2 agonists into the periaqueductal gray (Nogueira and Graeff, 1995; Zangrossi et al., 2001) and the tectum (Melo and Brandao, 1995) have been shown to decrease escape behavior and the aversiveness of electrical stimulation of the brain. Microinjections of kainic acid into the dorsal raphe, which elevate 5-HT levels in the amygdala and periaqueductal gray, decrease escape behavior in the T-maze (Graeff et al., 1997; Viana et al., 1997). In contrast, electrolytic or chemical lesions of the raphe, which reduce forebrain 5-HT levels, increase avoidance and escape behavior, death rate, and gastric ulceration, and decrease immunity (Andrade and Graeff, 2001; Andrade et al., 1999). The possibility that DOI injected into these areas might alter behaviors evoked by tail pinch stress remains to be investigated.

References

Alper RH. Hemodynamic and renin responses to (±)-DOI, a selective 5-HT_2 receptor agonist, in conscious rats. Eur J Pharmacol 1990; 175:323–32.

Anderson IK, Martin GR, Ramage AG. Central administration of 5-HT activates 5-HT_{1A} receptors to cause sympathoexcitation and 5-HT_2/5-HT_{1C} receptors to release vasopressin in anaesthetized rats. Br J Pharmacol 1992;107:1020–8.

Andrade TG, Graeff FG. Effect of electrolytic and neurotoxic lesions of the median raphe nucleus on anxiety and stress. Pharmacol Biochem Behav 2001;70:1–14.

Andrade TG, Silva AM, Silva CL, Graeff FG. Effect of electrolytic lesion of the median raphe nucleus on behavioral and physiological measures of stress. Acta Physiol Pharmacol Ther Latinoam 1999;49:279–89.

Angulo JA, Printz D, Ledoux M, McEwen BS. Isolation stress increases tyrosine hydroxylase mRNA in the locus coeruleus and midbrain and decreases proenkephalin mRNA in the striatum and nucleus accumbens. Brain Res Mol Brain Res 1991;11:301–8.

Bell AA, Butz BL, Alper RH. Cardiovascular responses produced by microinjection of serotonin-receptor agonists into the paraventricular nucleus in conscious rats. J Cardiovasc Pharmacol 1999;33:175–80.

Chaouloff F. Physiopharmacological interactions between stress hormones and central serotonergic systems. Brain Res Rev 1993;18:1–32.

Chaouloff F, Baudrie V, Coupry I. Effects of chlorisondamine and restraint on cortical [^{3}H]ketanserin binding, 5-HT$_{2A}$ receptor-mediated head shakes, and behaviours in models of anxiety. Neuropharmacology 1994;33:449–56.

Darmani NA, Shaddy J, Gerdes CF. Differential ontogenesis of three DOI-induced behaviors in mice. Physiol Behav 1996;60:1495–500.

Dey S. Physical exercise as a novel antidepressant agent: possible role of serotonin receptor subtypes. Physiol Behav 1994;55:323–9.

Gorzalka BB, Hanson LA, Brotto LA. Chronic stress effects on sexual behavior in male and female rats: mediation by 5-HT$_{2A}$ receptors. Pharmacol Biochem Behav 1998;61:405–12.

Graeff FG, Viana MB, Mora PO. Dual role of 5-HT in defense and anxiety. Neurosci Biobehav Rev 1997;21:791–9.

Graeff FG, Silva M, Del Ben CM, Zuardi AW, Hetem LA, Guimaraes FS. Comparison between two models of experimental anxiety in healthy volunteers and panic disorder patients. Neurosci Biobehav Rev 2001;25:753–9.

Granoff MI, Ashby CR. The effect of the repeated administration of the compound 3,4-methylenedioxymethamphetamine on the response of rats to the 5-HT$_{2A,C}$ receptor agonist (±)-1-(2,5-dimethoxy-4-iodophenyl-2-aminopropane) (DOI). Neuropsychobiology 1998;37:36–40.

Kaur P, Ahlenius S. Non-serotonergic potentiation by (−)-pindolol of DOI-induced forward locomotion in rats: possible involvement of β-adrenoceptors. J Neural Transm 2000;107:903–17.

Krebs-Thomson K, Geyer MA. The role of 5-HT$_{1A}$ receptors in the locomotor-suppressant effects of LSD: WAY-100635 studies of 8-OH-DPAT, DOI and LSD in rats. Behav Pharmacol 1996;7:551–9.

Kuroda Y, Mikuni M, Ogawa H, Takahashi K. Effect of ACTH, adrenalectomy and the combination treatment on the density of the 5-HT$_2$ receptor binding sites in neocortex of rat forebrain and 5-HT$_2$ receptor mediated wet-dog shake behaviors. Psychopharmacology 1992;108:27–32.

Lemoine AP, Armando I, Brun JC, Segura ET, Barontini M. Footshock affects heart and brain MAO and MAO inhibitory activity and open field behavior in rats. Pharmacol Biochem Behav 1990;36:85–8.

Lu CC, Tseng CJ, Wan FJ, Yin TH, Tung CS. Role of locus coeruleus and serotonergic drug actions on schedule-induced polydipsia. Pharmacol Biochem Behav 1992;43:255–61.

McCall RB, Clement ME. Role of serotonin$_{1A}$ and serotonin$_2$ receptors in the central regulation of the cardiovascular system. Pharmacol Rev 1994;46:231–43.

McKittrick CR, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR. Serotonin receptor binding in a colony model of chronic social stress. Soc Biol Psychiatry 1995;37:383–93.

Melo LL, Brandao ML. Role of 5-HT$_{1A}$ and 5-HT$_2$ receptors in the aversion induced by electrical stimulation of inferior colliculus. Pharmacol Biochem Behav 1995;51:317–21.

Mora PO, Netto CF, Graeff FG. Role of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes in the two types of fear generated by the elevated T-maze. Pharmacol Biochem Behav 1997;58:1051–7.

Nagayama H, Lu JQ. Circadian rhythm in the responsiveness of central 5-HT2A receptor to DOI in rats. Psychopharmacology 1996;127:113–6.

Nankai M, Yamada S, Muneoka K, Toru M. Increased 5-HT$_2$ receptor-mediated behavior 11 days after shock in learned helplessness. Eur J Pharmacol 1995;281:123–30.

Njung'e K, Handley SL. Effects of 5-HT uptake inhibitors, agonists and antagonists on the burying of harmless objects by mice; a putative test for anxiolytic agents. Br J Pharmacol 1991;104:105–12.

Nogueira RL, Graeff FG. Role of 5-HT receptor subtypes in the modulation of dorsal periaqueductal gray generated aversion. Pharmacol Biochem Behav 1995;52:1–6.

Olivier B, Molewijk HE, van der Heyden JA, van Oorschot R, Ronken E, Mos J, Miczek KA. Ultrasonic vocalizations in rat pups: effects of serotonergic ligands. Neurosci Biobehav Rev 1998;23:215–27.

Pellegrino LJ, Pellegrino AS, Cushman AJ. A stereotaxic atlas of the rat brain. New York: Plenum, 1979.

Raghavendra V, Kulkarni SK. Melatonin reversal of DOI-induced hypophagia in rats; possible mechanism by suppressing 5-HT$_{2A}$ receptor mediated activation of the HPA axis. Brain Res 2000;860:112–8.

Redrobe JP, Bourin M. Partial role of 5-HT$_2$ and 5-HT$_3$ receptors in the activity of antidepressants in the mouse forced swimming test. Eur J Pharmacol 1997;325:129–35.

Sanchez C, Mork A. N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline studies on the role of 5-HT$_{1A}$ and 5-HT$_2$ receptors in mediating footskock-induced ultrasonic vocalisation in adult rats. Eur Neuropsychopharmacol 1999;9:287–94.

Silva M, Hetem LA, Guimaraes FS, Graeff FG. Opposite effects of nefazodone in two human models of anxiety. Psychopharmacology 2001;156:454–60.

Torda T, Culman J, Cechova E, Murgas K. 3-H-Ketanserin (serotonin type 2) binding in the rat frontal cortex: effect of immobilization stress. Endocrinol Exp 1988;22:99–105.

Viana MB, Graeff FG, Loschmann PA. Kainate microinjection into the dorsal raphe nucleus induces 5-HT release in the amygdala and periaqueductal gray. Pharmacol Biochem Behav 1997;58:167–72.

Welch JE, Saphier D. Central and peripheral mechanisms in the stimulation of adrenocortical secretion by 5-hydroxytryptamine$_2$ agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane. J Pharmacol Exp Ther 1994;270:918–28.

Yamada S, Watanabe A, Nankai M, Toru M. Acute immobilization stress reduces (+/−)DOI-induced 5-HT2$_A$ receptor-mediated head shakes in rats. Psychopharmacology 1995;119:9–14.

Zangrossi H, Viana MB, Zanoveli J, Bueno C, Nogueira RL, Graeff FG. Serotonergic regulation of inhibitory avoidance and one-way escape in the rat elevated T-maze. Neurosci Biobehav Rev 2001;25:637–45.