*Pharmacology Biochemistry and Behavior*, Vol. 41, pp. 581–585, 1992
Printed in the U.S.A. 

0091-3057/92 $5.00 + .00


# Effects of Eltoprazine Hydrochloride on Reactivity to Conspecific or Novel Odors and Activity

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Received 23 August 1991

OSTREM, J. L., J. M. RAWLEIGH AND E. D. KEMBLE. *Effects of eltoprazine hydrochloride on reactivity to conspecific or novel odors and activity.* PHARMACOL BIOCHEM BEHAV **41**(3) 581–585, 1992.—Treatment with eltoprazine (DU 28853) increased the number of entries by male mice into compartments containing the odors of male and female conspecifics. This effect was most pronounced when odors were provided by previously defeated males. In contrast, the drug had no effect upon responsiveness to the odors of cinnamon and chocolate. The results suggest that eltoprazine may selectively increase reactivity to conspecific odors and that this effect is further enhanced by agonistic experience. Eltoprazine also substantially increased activity levels in all experiments. Since hyperactivity occurred both in the presence and absence of conspecific odors, however, the drug's effects on activity and olfaction seem to be largely independent. The results suggest that the aggression-modulating effects of eltoprazine, as well as other drugs, may be mediated in part by their effects on normal olfactory function.

Eltoprazine hydrochloride (DU 28853) Olfactory preference Conspecific odors Submissive odors Novel odors Activity

A recently synthesized family of phenylpiperazine compounds (DU 27716, 27725, 28412) strongly inhibits offensive attack behavior in a wide range of testing conditions while having no effect upon, or increasing, defense. Treatment with these serotonergic agonists also often increases conspecific sniffing behavior [e.g., (1,2,7,15–18,20)], which has been interpreted as reflecting a rather general increase in social interest [e.g., (16–19)]. Alternatively, however, the fact that the odors of dominant conspecifics or potential (cat) predators suppress attack behavior and induce hypoalgesia (23,28,29) suggests that the drugs may have a more primary effect on olfactory processes. Recently, both DU 27716 (fluprazine) and yohimbine have been shown to increase preference for the odors of conspecific males (10,12) and fluprazine to impair performance of a food-rewarded olfactory discrimination (27). These results suggest that drugs, differing markedly in their pharmacological activities, may share olfactory effects in common. The present experiments explored the possible olfactory actions of a recently synthesized phenylpiperazine (eltoprazine, DU 28853). Like other members of this drug family, eltoprazine selectively inhibits attack behavior and increases conspecific sniffing (18,19) but does not seem to exhibit the rather uniform fear-potentiating effects of fluprazine (11,13). It therefore seemed of interest to examine the effects of eltoprazine on responsiveness to conspecific odors. Since previous research has shown that both familiarity and previous stress experiences alter the olfactory preferences of rats and mice [e.g., (3,4,24,26)], the odors of conspecific males previously defeated during a series of agonistic encounters were included, as well as those of unfamiliar males and females.

## GENERAL METHOD

### *Subjects*

Experimentally naive male CD-1 mice weighing 28.9–42.2 g served as subjects. Mice were reared with littermates until 24–28 days of age, housed in like-sex groups of 6–8 for an additional 28–32 days, and individually housed for 21 days prior to testing. All mice were housed on a sawdust substrate with a 12 L:12 D cycle with all testing conducted during the light phase. The mice had ad lib access to Purina Lab Chow and water throughout testing.

### *Apparatus*

Testing was conducted in a four-choice olfactory preference apparatus. The apparatus consisted of a 30 × 30 × 17.5 cm central chamber that allowed access to four 20 × 11.5 × 17.5 cm odor compartments via sliding guillotine doors placed

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in the center of each central chamber wall. The walls of the apparatus were of white Plexiglas and the ceiling and guillotine doors of clear Plexiglas. The floor of the central chamber and adjacent 10 cm of each odor compartment were of black Plexiglas. The most distal 10 cm of each odor compartment floor consisted of an aluminum plate containing 84 evenly spaced 0.4-mm perforations. The apparatus floor was elevated by 4.5 cm and a 10 × 9 cm Plexiglas tray containing the odorous substances was inserted under the perforated aluminum floor. Further isolation of odors to the odor compartment was provided by a Plexiglas baffle that extended beneath the apparatus floor at the end of each aluminum plate most proximal to the central chamber. The apparatus and odor trays were thoroughly cleaned with a detergent solution after each trial and the positions of the odors systematically varied among subjects. The apparatus was placed in a sound-attenuating room under dim (85 W) red illumination. Observations were carried out from an adjacent room through a one-way vision screen.

*Procedure*

All subjects were adapted to the apparatus with no odors present for 30 min. Two days later, mice were randomly assigned to weight-balanced groups designated to receive 0.5-mg/kg (Low) or 2.0-mg/kg (High) dosages of eltoprazine or an equivalent volume of isotonic saline (Saline) by intraperitoneal injection. These eltoprazine dosages are well below sedative ataxic levels and markedly suppress attack behavior (16,18). Following injection, mice were returned to their home cages. Thirty min later, odorous materials were placed in the odor trays and placed under the floors of the odor compartments. Subjects were then gently placed in the center compartment with the doors to all odor compartments closed. All (Experiments 1 and 3) or three (Experiment 2) of the doors to the odor compartments were opened simultaneously 45–60 s later. Number of entries into each odor compartment and total time spent in each compartment were recorded during a 10-min trial. Odorous materials were immediately removed from the apparatus at the end of each trial and the apparatus thoroughly cleaned. Fresh odorous material was used for each trial.

## EXPERIMENT 1

Experiment 1 examined the effects of eltoprazine on responsiveness to conspecific odors. Odors of unfamiliar males and females were provided to detect any generalized shift in responsiveness, while odors of previously defeated males were utilized to explore any possible preference shifts produced by agonistic experience.

### METHOD

*Subjects*

Subjects were 32 male mice (residents) designated to receive Low ($n$ = 10) or High ($n$ = 10) eltoprazine or Saline ($n$ = 12) doses. Submissive odors were provided by 10 male mice (intruders) weighing 5.5–6.4 g less than residents. Unfamiliar male odors were provided by 12 mice housed in groups of 6. Unfamiliar female odors were provided by eight individually housed mice. The amount of sawdust bedding per mouse was equal for group and individually housed mice to assure approximately equal concentrations of conspecific odors. Conspecific odors were allowed to accumulate in the bedding for 21 days prior to preference testing.

*Procedure*

Residents were individually housed for 21 days. At the end of this time, the smaller intruders were placed in the resident's home cage on three consecutive days (day 1, 10 min, days 2–3, 5 min). All residents promptly attacked intruders with species-typical offensive behaviors to which all intruders responded defensively. During this time, residents were also adapted to the apparatus. Preference testing was conducted 48 h after the last resident-intruder encounter. Following drug or saline injection, and immediately prior to testing, 100 ml of submissive, unfamiliar male, unfamiliar female, and unsoiled sawdust bedding were inserted beneath the floors of the four odor compartments. Resident mice then received a 10-min preference test.

### RESULTS

Overall drug effects were assessed by separate single-factor analyses of variance (ANOVA's) for each of the four odors and individual comparisons made by Scheffe's tests. Mean time spent in each of the four odor compartments is summa-

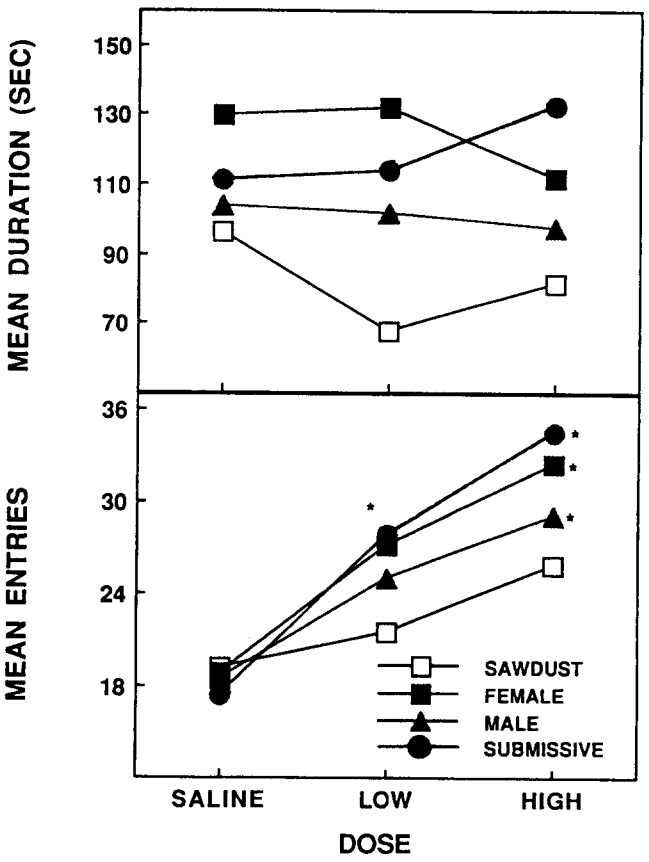


FIG. 1. Mean duration of contact (upper panel) with, and number of entries into (lower panel), compartments containing the odors of submissives, unfamiliar males and females, and sawdust by eltoprazine- and saline-treated male mice. Asterisks indicate significant differences from saline levels ($p < 0.05$).

rized in the upper panel of Fig. 1. Duration of contact with unfamiliar male and unfamiliar female odors were similar for all groups, $F$'s $< 1.08$, $p$'s $> 0.10$. Although eltoprazine increased duration of contact with submissive odors, these differences only approached statistical significance, $F(2,28) = 2.68$, $p < 0.10$, and appeared to be restricted to the highest dosage. The Low ($p < 0.05$) but not High ($p > 0.10$) eltoprazine dose also decreased duration in the sawdust compartment.

Numbers of entries into each of the four odor compartments are summarized in the lower panel of Fig. 1. Drug treatment produced a highly significant elevation of entries into the submissive compartment, $F(2,28) = 12.66$, $p < 0.001$, with both Low and High dosages differing significantly from the Saline Group ($p$'s $< 0.05$) but not from each other ($p > 0.10$). Unfamiliar male, $F(2,28) = 4.90$, $p < 0.02$, and unfamiliar female, $F(2,28) = 9.28$, $p < 0.001$, entries were significantly elevated at the High ($p$'s $< 0.05$), but not Low ($p$'s $> 0.10$) dose. Drug treatment also induced a marginally significant elevation in sawdust entries, $F(2,28) = 2.95$, $p < 0.10$. As can be seen in Fig. 1, eltoprazine also seemed to substantially increase number of compartment entries regardless of the odor present. Entries into all four compartments were therefore combined to yield an overall index of activity. Eltoprazine produced a highly significant increase, $F(2,28) = 13.49$, $p < 0.001$, in total compartment entries with both Low and High Groups differing significantly from Saline ($p$'s $< 0.05$) but not from each other ($p > 0.10$). Durations of contact with all four odors combined, however, revealed closely similar means (means = 418–440 s) with no suggestion of group differences ($F < 1.0$).

## EXPERIMENT 2

Experiment 1 indicates that eltoprazine enhances responsiveness to conspecific odors and suggests that it may have its strongest effects on odors associated with prior agonistic encounters. Since number of entries into all compartments, including sawdust, was increased by the drug, it seemed possible that drug effects might extend to nonconspecific odors as well. Experiment 2 therefore explored the effects of eltoprazine on responsiveness to the odors of chocolate and cinnamon. These odors have been repeatedly shown to be effective for the social transmission of food selection, which is importantly mediated by olfaction [e.g., (8)], but are strongly rejected in preference tests (22). If eltoprazine has generalized effects on olfaction, some amelioration of this rejection might be expected.

### METHOD

Thirty male mice were designated to receive Low ($n = 10$) or High ($n = 10$) dosages of eltoprazine or Saline ($n = 10$). Subjects were individually housed for 5 days, adapted to the apparatus, and received the appropriate drug or saline injections 30 min prior to placement in the preference apparatus. Immediately prior to testing, 0.65 g Hershey's unsweetened chocolate was placed beneath one odor compartment, 0.65 g cinnamon (Trader's Choice) under a second, and 8.65 g unsoiled sawdust beneath the third. The guillotine door to the fourth compartment remained closed during this test.

### RESULTS

The results of Experiment 2 are summarized in Fig. 2. It can be seen (upper panel) that all groups spent less time

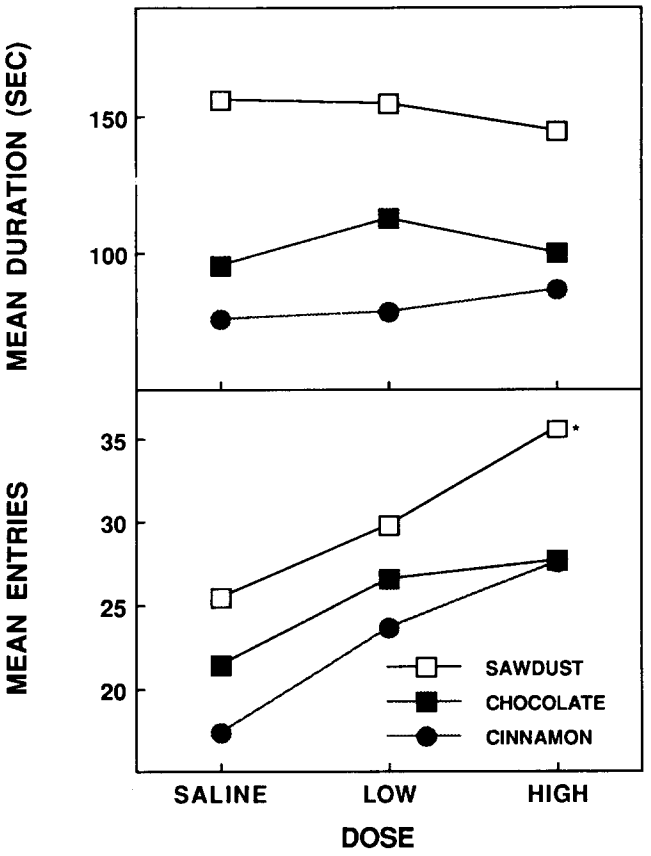


FIG. 2. Mean duration of contact (upper panel) with, and number of entries into (lower panel), compartments containing the odors of cinnamon, chocolate, or sawdust by eltoprazine- and saline-treated male mice. Asterisks indicate significant differences from saline levels ($p < 0.05$).

in contact with chocolate and cinnamon odors than sawdust ($p$'s $< 0.001$) but with no suggestion of a drug effect (all $F$'s $< 1.0$). Eltoprazine (lower panel, Fig. 2) increased entries into the sawdust compartment, $F(2,27) = 3.73$, $p < 0.05$, at the High ($p < 0.05$) but not Low ($p > 0.10$) dose. There were no drug effects on entries into either chocolate or cinnamon compartments, however ($p$'s $> 0.10$). As in Experiment 1, eltoprazine seemed to increase overall activity levels. Total compartment entries were significantly elevated, $F(2,27) = 3.60$, $p < 0.05$, by eltoprazine with the High ($p < 0.05$) but not Low ($p > 0.10$) dose differing significantly from the Saline Group. Mean total duration of contact with all odors combined (means = 327–347 s) was similar among all groups with no suggestion of a drug effect, $F < 1.0$.

## EXPERIMENT 3

Although the increases in total compartment entries noted in the previous experiments indicate that eltoprazine produces hyperactivity, it remains possible that this effect is secondary to a rather diffuse increase in responsiveness to odors. If so, drug-induced hyperactivity might be expected to diminish or disappear in the absence of salient conspecific or novel odors. Experiment 3 examined total compartment entries in the preference apparatus when all four compartments contained only the odor of unsoiled sawdust bedding. Since drug effects on

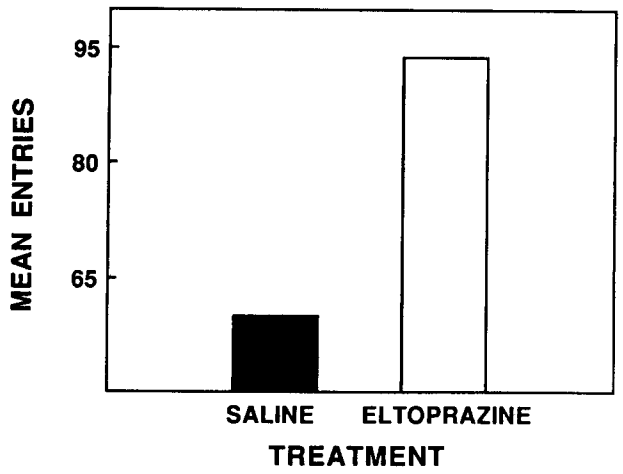


FIG. 3. Mean total entries into sawdust compartments by eltoprazine- and saline-treated male mice.

activity were most pronounced at the highest dosage in Experiment 1 and 2, only the high dose was utilized for this experiment.

### METHOD

Twenty male mice received either the High ($n$ = 10) dose of eltoprazine or Saline ($n$ = 10). Mice were tested as described in Experiment 1 but with unsoiled sawdust bedding placed beneath each of the four compartments. Only number of compartment entries were recorded in this experiment.

### RESULTS

The results of this experiment are summarized in Fig. 3. It can be seen that mean entries by the Drug Group (mean = 93.7) was more than 50% higher than that of the Saline Group (mean = 59.9), with the highest number of Saline entries ($n$ = 86) overlapping with only the three lowest scoring drug-treated mice ($n$'s = 46–82). Comparison by $t$-test revealed a highly significant group difference, $t(18) = 3.67, p < 0.002$.

## GENERAL DISCUSSION

The present results indicate that the antiaggressive actions of eltoprazine are accompanied by increased responsiveness to conspecific odors. Although the substantial increase in activity induced by eltoprazine somewhat complicates interpretation, the fact that eltoprazine induced hyperactivity in the absence of conspecific odors (Experiment 3) argues that increased activity was not secondary to the drug's olfactory effects. The increase in novel open-field entries and maze exploration and decreased latencies to approach and contact a novel object observed in previous studies (11) are also consistent with this interpretation. These findings thus provide a suggestive parallel with the effects of yohimbine and fluprazine, both of which inhibit attack (1,7,10), increase conspecific sniffing (10,19), and alter conspecific olfactory preference (10, 12). Dixon (5) has also shown that resident mice show an increase in attack behavior when intruders are treated with the urine of diazepam-treated mice, presumably reflecting a drug-induced change in the olfactory properties of the urine. Further, scopolamine not only decreases play (26) and aggressive behavior [e.g., (9,14)] but also alters familiarization with juvenile conspecific odors (25). Taken together, the data suggest that altered olfactory responsiveness may be a rather common effect of aggression-modulating drugs having diverse pharmacological properties. Since the olfactory investigation of intruding rodents is a near-invariable prelude to attack by residents, a drug-induced intensification of this behavior may substantially reduce attack by either directly inhibiting it or by increasing the tendency of intruders to attack, thus necessitating increased defense by residents. Indeed, the latter possibility is suggested to account for the increase in defensive behavior sometimes seen among animals treated with various phenylpiperazine compounds [e.g., (15,17)]. Of particular interest, the present data also suggest that eltoprazine's olfactory effects may be exaggerated by agonistic experience. Although clearly speculative, the differential responsiveness of mice and rats to familiar or stressed conspecific odors [e.g., (3,4,24)] and the suppression of attack, increased freezing, and hypoalgesia induced by the odors of dominant conspecifics and potential predators (23,28,29) lend plausibility to this suggestion. Alternatively, however, the fact that resident mice in Experiment 1 had social contact only with submissives raises the possibility that enhanced preference for their odors was due to prior familiarization. Further studies that compare the reactivity to the odors of familiar nonaggressive conspecifics (e.g., littermates) with those of submissives would be useful in addressing this issue.

Although the above data indicate that at least several antiaggressive drugs also have some olfactory effects in common, it must be emphasized that these effects differ in potentially important ways. Fluprazine increases preference for male, but not female or food odors over a 15-min trial (12), impairs olfactory discrimination (27), but has no effect on odor detection thresholds (6). Yohimbine, in contrast, produces a transitory decrease in preference for male odors (10) and has no effect on olfactory discrimination (21), while scopolamine alters responsiveness to the odors of familiar but not unfamiliar juveniles (25). Further systematic research is clearly needed to reveal similarities and differences in the olfactory effects of drugs sharing similar antiaggressive actions. The fact that fluprazine alters conspecific odor preference (12) but not olfactory thresholds (6), the differential effect of scopolamine on odors of familiar but not unfamiliar juveniles (25), and the failure of eltoprazine to alter responsiveness to novel odors (Experiment 2) suggest that the drugs are most likely to impair more complex olfactory processes. Further studies of such drug effects on olfaction are currently in progress.

### ACKNOWLEDGEMENT

The authors thank Duphar B. V. Weesp, who kindly supplied the eltoprazine used in these experiments.

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