Psychopharmacology (1990) 102:498–502

Psychopharmacology

Serenics fluprazine (DU 27716) and eltoprazine (DU 28853) enhance neophobic and emotional behaviour in mice

Guy Griebel[1], Martine Saffroy-Spittler[1], René Misslin[1], Elise Vogel[1], and James R. Martin[2]

[1] Laboratoire de Psychophysiologie, 7 rue de l'Université, F–67000 Strasbourg, France
[2] F. Hoffmann-La Roche Ltd., Pharmaceutical Research Department, CH–4002 Basel, Switzerland

Received April 26, 1990 / Final version June 15, 1990

Abstract. Two tests designed to elicit responses to novelty and to aversive stimuli were used to study the effects of the serenics fluprazine and eltoprazine on the behaviour of male Swiss mice: a free exploratory test (fluprazine: 2.5, 5 and 10 mg/kg; eltoprazine: 2.5, 5, 10 and 15 mg/kg) and a two-box choice procedure (fluprazine: 1.25, 2.5, 5 and 7.5 mg/kg; eltoprazine: 2.5, 5, 7.5 and 10 mg/kg). Both drugs increased the neophobic reaction, as well as the avoidance of a brightly illuminated box. These effects closely resemble those of psychostimulant drugs such as methamphetamine and caffeine. It is hypothesized that the behavioural changes induced by these drugs may be due to a nonspecific increase of the emotional reactivity of animals.

Key words: Fluprazine – Eltoprazine – Serenics – Exploration – Locomotion – Rearing – Light/dark procedure – Anxiety – Emotionality – Mice

Olivier et al. (1986) have provided evidence for the antiaggressive effects of a class of drugs termed serenics. These compounds specifically inhibit offensive aggression in rats (Olivier 1980; Muraoka et al. 1983; Racine et al. 1984; Flannelly et al. 1985; Olivier et al. 1985) and mice (Benton et al. 1983; Brain et al. 1983; Olivier et al. 1989; Parmigiani et al. 1989), while sometimes increasing various aspects of defensive behaviour (Brain et al. 1983; Benton et al. 1983). However, Pool et al. (1986) suggested that fluprazine does not selectively inhibit offensive aggression in rats. Furthermore, it has been shown that increased fear is a potent inhibitor of offense, while defensive behaviour often occurs in situations involving fear and anxiety (Blanchard et al. 1985, 1989). Indeed, some data strongly suggest that an increase of fearfulness induced by serenics underlies their so-called selective antiaggressive properties (Schultz and Kemble 1986; Kemble et al. 1987; Kemble 1989). To examine this hypothesis, the effects of several doses of fluprazine (DU 27716) and eltoprazine (DU 28853) were investigated on the behaviour of mice placed in a free exploratory situation as described by Hughes (1965) and adapted for use in mice by Misslin and Ropartz (1981), as well as in the light/dark choice test described by Crawley and Goodwin (1980) and modified by Misslin et al. (1989). In the first test, psychostimulant drugs such as amphetamine, methylphenidate and caffeine have been found to enhance neophobia (Hughes 1972; Hughes and Greig 1976; Misslin and Ropartz 1981), while in the second procedure several benzodiazepine receptor antagonists and inverse agonists β-carboline, flumazenil, RO 15–3505, RO 15–4513) have been reported to potentiate anxious responses towards aversive stimuli (Belzung et al. 1987, 1988a, b).

Offprint requests to: R. Misslin

Materials and methods

Animals

Male Swiss albino mice from the Laboratoire de Psychophysiologie (Strasbourg), 12 weeks old at time of testing, were used. Prior to experimental testing, they were housed five to a standard cage containing a constant supply of food pellets and water, and kept on a 12/12-h light-dark cycle with light onset at 0100 hours. The mice were tested during the dark phase.

Drugs

Fluprazine and eltoprazine (synthesized by Dr. Jakob-Roetne. Hoffmann-La Roche, Basel) were dissolved in saline and administered intraperitoneally, 30 min before testing, at different concentrations in a volume of 10 ml/kg body wt.

Experiment 1

Apparatus. The apparatus consisted of a polyvinyl box (30 × 20 × 20 cm) subdivided into six equal, square units and covered with Plexiglas.

It could be temporarily divided in half by means of three partitions. The apparatus was located on a stand in the room in which the mice were housed. During testing, the experimenters always stood at the same place next to the test apparatus.

Procedure. Each mouse was placed for approximately 24 h in one half of the apparatus with the temporary partitions inserted. This permitted familiarization with the test situation. Approximately 24 h after this familiarization, each mouse was exposed to both the familiar and novel environments by the removal of the temporary partitions. The subject was then observed in red light for 10 min. The time spent by mice in the novel compartment (novelty preference), the number of units entered by the subject (locomotion) and the number of rearing responses were recorded.

Mice were randomly allocated to the following groups: a) fluprazine: vehicle control (saline; $n=10$) and drug (2.5, 5 and 10 mg/kg in saline; $n=10$); b) eltoprazine: vehicle control (saline; $n=10$) and drug (2.5, 5, 10 and 15 mg/kg in saline; $n=10$).

Experiment 2

Apparatus. The apparatus consisted of two polyvinyl chloride boxes (20 × 20 × 14 cm) covered with Plexiglas. One of these boxes was kept darkened. A light from a 100-W desk lamp above the other provided the only room illumination. An opaque plastic tunnel (5 × 7 × 10 cm) separated the dark box from the illuminated one. During testing the experimenter always sat at the same place next to the apparatus.

Procedure. The subjects were individually tested in 5-min sessions in the apparatus described above. All mice were placed in the illuminated box to initiate the test session. The amount of time spent by mice in the illuminated box and the number of tunnel crossings were recorded minute by minute, during 5 min after the first entry into the dark box. A mouse whose four paws were in the new box was considered to have changed boxes. Mice were randomly allocated to the following groups: a) fluprazine: vehicle control (saline; $n=20$) and drug (1.25, 2.5, 5, 7.5 mg/kg in saline; $n=20$); b) eltoprazine: saline control (saline; $n=20$) and drug (2.5, 5, 7.5 and 10 mg/kg in saline; $n=20$). The latter dose range was determined by the marked behavioural disorganization observed at high doses in experiment 1.

Statistical Analysis

Statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance followed by the Dunnett test or, in the case of unequal variances, by the Bonferroni test.

Results

Experiment 1

Analysis of variance revealed significant differences among the groups with respect to the novelty preference [fluprazine: $F(3,36)=9.68$, $P<0.001$; eltoprazine: $F(4,45)=21$, $P<0.001$], locomotion [fluprazine: $F(3,36)=5.69$, $P<0.001$; eltoprazine: $F(4,45)=15$, $P<0.001$] and rearing behaviour [fluprazine: $F(3,36)=8.40$, $P<0.001$; eltoprazine: $F(4,45)=6.63$, $P<0.001$]. Figures 1 and 2 show that the drugs induced a dose-dependent decrease in the novelty preference, as well as in the rearing behaviour, while they increased the locomotion at the low and intermediate doses. This latter effect was significant only with eltoprazine.

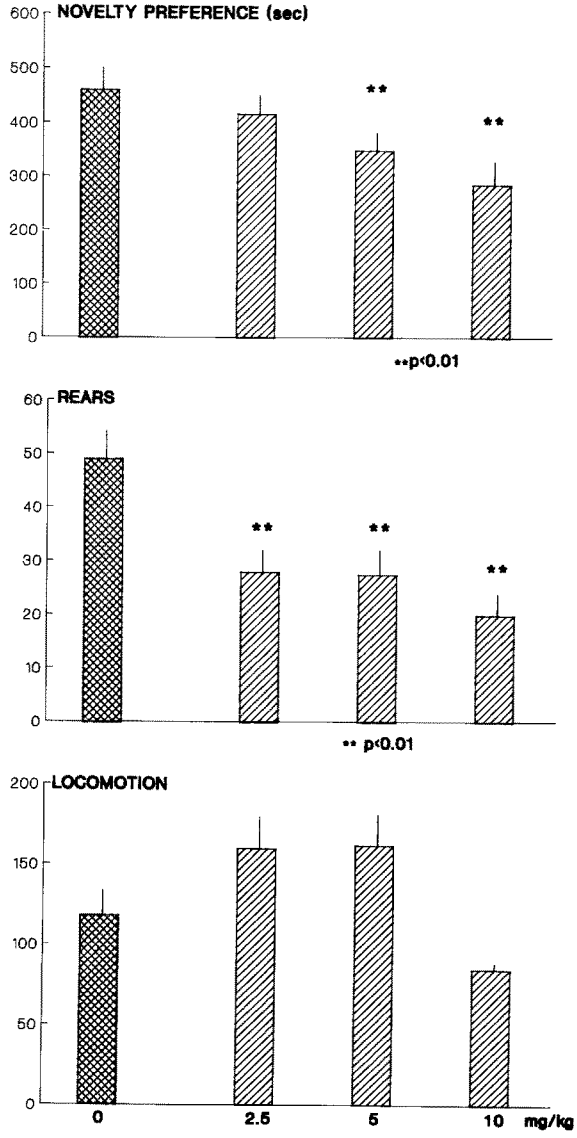

Fig. 1. Effects of fluprazine on several behavioural responses (means ± SEM) of mice in a free exploration situation

Experiment 2

Analysis of variance revealed significant differences between groups with respect to the time spent by mice in the illuminated box [fluprazine: $F(4,95)=2.67$, $P<0.03$; eltoprazine: $F(4,95)=2.84$, $P<0.02$] and to the number of tunnel crossings [fluprazine: $F(4,95)=8.09$, $P<0.001$; eltoprazine: $F(4,95)=4.7$, $P<0.001$]. Figures 3 and 4 show that the drugs tended to decrease both of these behavioural variables.

Discussion

The data obtained here support the hypothesis that the selective antiaggressive properties reported for the

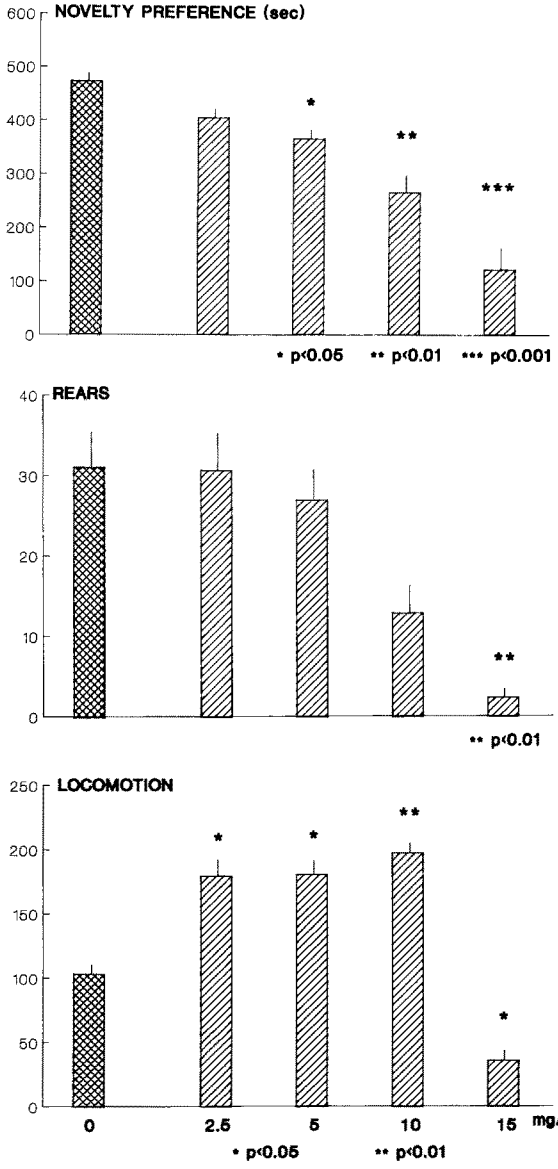

Fig. 2. Effects of eltoprazine on several behavioural responses (means ± SEM) of mice in a free exploration situation

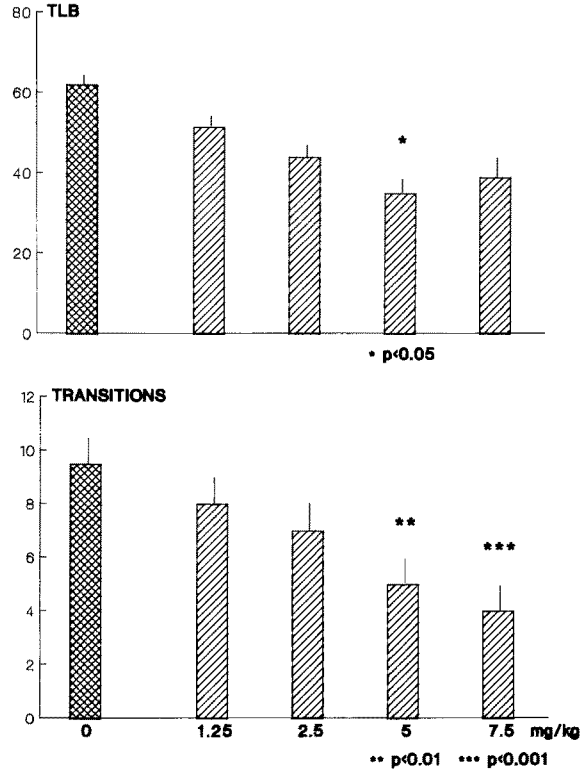

Fig. 3. Effects of fluprazine on the time spent by mice in the illuminated box (TLB: means ± SEM) and on the number of tunnel crossings from the illuminated box to the dark one (transitions) (means ± SEM)

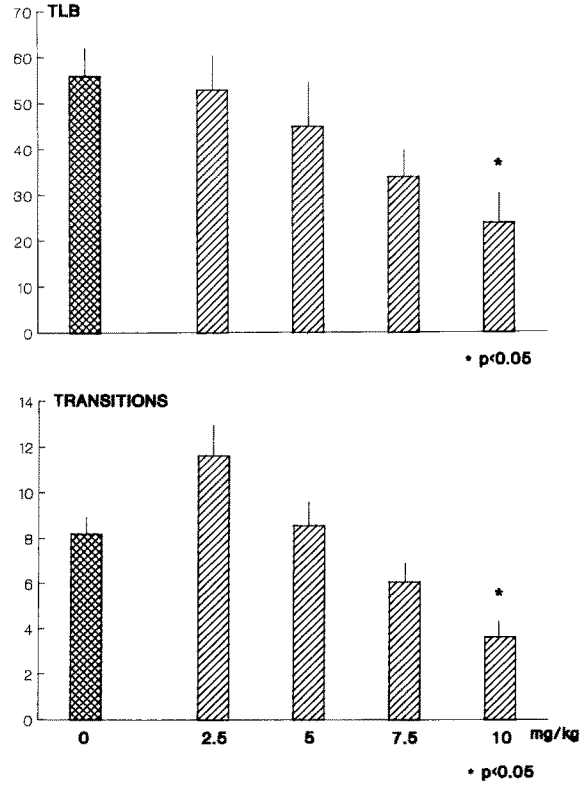

Fig. 4. Effects of eltoprazine on the time spent by mice in the illuminated box (TLB: means ± SEM) and on the number of tunnel crossings from the illuminated box to the dark one (transitions) (means ± SEM)

phenylpiperazines fluprazine and eltoprazine (Olivier 1980; Benton et al. 1983; Brain et al. 1983; Muraoka et al. 1983; Racine et al. 1984; Flannelly et al. 1985; Blanchard et al. 1985; Olivier et al. 1985, 1989) are probably due, at least in part, to their ability to increase fear and emotionality, as has been previously suggested by other authors (Schultz and Kemble 1986; Kemble et al. 1987; Kemble 1989). We examined this hypothesis in both a free exploration situation and in a light/dark choice procedure which have been behaviourally validated for detecting anti-anxiety effects (Misslin et al. 1989). Experiment 1 indicates that fluprazine and eltoprazine selectively reduced exploratory parameters such as the time spent by mice in a novel compartment and the number of rearing responses, while they tended to increase locomotion, at least at the low and intermediate doses. These findings closely parallel those which were found for methamphetamine in the same test (Misslin and Ropartz

1981). It has previously been reported that several psychostimulant drugs such as amphetamine, methylphenidate and caffeine increased locomotor activity and decreased exploration (Hughes 1972; Robbins and Iversen 1973; Hughes and Greig 1976). It is hypothesized that these drugs are able to simultaneously stimulate locomotor activity and to increase emotional defence reactions towards novelty (neophobia). Experiment 2 demonstrates that the two serenics tended to increase avoidance responses towards the brightly illuminated box insofar as they reduced the time spent by mice in the illuminated box as well as the number of crossings between the illuminated box and the dark one. These results resemble those observed after administration of inverse agonists at the benzodiazepine receptor (Belzung et al. 1987, 1988a, b). This latter effect, which appears to be opposite to that of anxiolytic properties of minor tranquilizers, has been considered as an anxiogenic action (File et al. 1982; Pellow and File 1986). Several psychostimulant drugs have also been reported to have "anxiogenic" effects, including caffeine (File and Hyde 1979; Baldwin et al. 1986, 1989; File et al. 1988) and methamphetamine (Belzung 1988 unpublished results). Taken together, the present findings demonstrate that the serenics fluprazine and eltoprazine have a general stimulating action, since they were able to enhance the locomotor activity of mice as well as their avoidance responses towards novelty and aversive stimuli, suggesting that these drugs may induce their reported selective antiaggressive effects by a nonspecific increase of emotionality. Indeed, it has been claimed that these drugs selectively inhibit offensive aggression in rats and mice while having no effect in rats on defensive behaviours (Blanchard et al. 1985) or dramatically increasing various aspects of defence in mice (Benton et al. 1983; Olivier et al. 1989). Interestingly, Benton et al. (1983) noted that in mice treated with fluprazine fewer offensive responses were associated with defensive and avoidance responses towards the opponent and that fluprazine tended to increase emotionality. In addition, Kemble et al. (1987) found that fluprazine enhanced the reactivity of mice towards both living and inanimate sources of novelty. In contrast, in rodents benzodiazepines have consistently been found to decrease defensive reactions induced by various stimuli (see Treit 1985 for a recent review).

References

Baldwin HA, File S (1989) Caffeine-induced anxiogenesis: the role of adenosine, benzodiazepine and noradrenergic receptors Pharmacol Biochem Behav 32:181–186

Baldwin HA, File SD, Johnston AL, Wilks W (1986) An investigation of the acute, chronic and withdrawal effects of caffeine on anxiety, exploration and convulsions in the rat. Soc Neurosci Abstr 12:906

Belzung C, Misslin R, Vogel E, Dodd RH, Chapouthier G (1987) Anxiogenic effects of methyl-β-carboline in a light/dark choice situation. Pharmacol Biochem Behav 28:29–33

Belzung C, Misslin R, Vogel E (1988a) The benzodiazepine receptor inverse agonists β-CCM and RO 15–3505 both reverse the anxiolytic effects of ethanol in mice. Life Sci 42:1765–1772

Belzung C, Misslin R, Vogel E (1988b) Does RO 15–4513 reverse the anxiolytic effects of ethanol by its intrinsic properties? Pharmacol Biochem Behav 30:867–870

Benton D, Brain PF, Jones S, Colebrook E, Grimm V (1983) Behavioural examinations of the anti-aggressive drug fluprazine. Behav Brain Res 10:325–338

Blanchard DC, Takushi R, Blanchard RJ, Flannelly KJ, Kemble ED (1985) Fluprazine hydrochloride does not decrease defensive behaviors of wild and septal syndrome rats. Physiol Behav 35:349–353

Blanchard RJ, Blanchard DC, Hori K (1989) An ethoexperimental approach to the study of defense. In: Blanchard RJ, Brain PF, Blanchard DC, Parmigiani S (eds) Ethoexperimental approaches to study of behavior. Kluwer, London, New York, pp 114–136

Brain PF, Jones SE, Kamis A (1983) A preliminary ethological analysis of the effects of DU27716 on intermale aggression in Swiss-Webster mice. Aggress Behav 9:117

Crawley JN, Goodwin FK (1980) Preliminary report of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol Biochem Behav 13:167–170

File SE, Hyde JRG (1979) A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilizers and stimulants. Pharmacol Biochem Behav 11:65–69

File SE, Bond AJ, Lister RJ (1982) Interaction between effects of caffeine and lorazepam in performance tests and self-ratings. J Clin Psychopharmacol 2:102–106

File SE, Baldwin HA, Johnston AL, Wilks LJ (1988) Behavioral effects of acute and chronic administration of caffeine in the rat. Pharmacol Biochem Behav 30:809–815

Flannelly KJ, Muraoka MY, Blanchard DC, Blanchard RJ (1985) Specific anti-aggressive effects fluprazine hydrochloride. Psychopharmacology 87:86–89

Hughes RN (1965) Food deprivation and locomotor exploration in the white rat. Anim Behav 13:30–32

Hughes RN (1972) Methylphenidate induced inhibition of exploratory behaviour in rats. Life Sci 11:161–167

Hughes RN, Greig AM (1976) Effects of caffeine, methamphetamine and methylphenidate on reactions to novelty and activity in rats. Neuropharmacology 15:673–676

Kemble ED (1989) Some further ethoexperimental studies of the anti-aggressive drug fluprazine hydrochloride. In: Blanchard RJ, Brain PF, Blanchard DC, Parmigiani S (eds) Ethoexperimental approach to the study of behavior. Kluwer, London, New York, pp 484–493

Kemble ED, Thornton AE, Schultz LA (1987) Some fear-potentiating effects of fluprazine hydrochloride in mice. Aggress Behav 13:269–280

Misslin R, Ropartz P (1981) Effects of methamphetamine on novelty-seeking behaviour by mice. Psychopharmacology 75:39–43

Misslin R, Belzung C, Vogel E (1989) Behavioural validation of a light/dark choice procedure for testing anti-anxiety agents. Behav Proc 18:119–132

Muraoka MY, Flannelly KJ, Blanchard DC, Blanchard RJ (1983) The effects of DU27716 on aggression and social behavior in the rat. Aggress Behav 9:118

Olivier B (1980) A new anti-aggressive compound. Ethological studies. Aggres Behav 6:262–263

Olivier B, Mos J, van Oorschot R (1985) Maternal aggression in rats: effects of chlordiazepoxide and fluprazine. Psychopharmacology 86:68–76

Olivier B, van Dalen D, Hartog J (1986) A new class of psychoactive drugs, serenics. Drugs of the Future 11:473–499

Olivier B, Mos J, van der Heyden J, Hartog J (1989) Serotonergic modulation of social interactions in isolated male mice. Psychopharmacology 97:154–156

Parmigiani S, Rodgers RJ, Palanza P, Mainardi M, Brain PF (1989) The inhibitory effects of fluprazine on parental aggression in female mice are dependent upon intruder sex. Physiol Behav 46:455–459

Pellow S, File SE (1986) Anxiolytic and anxiogenic drug effects on

exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 24:525–529

Racine M, Flannelly KJ, Blanchard DC (1984) Antiaggressive effects of DU27716 on attack and defensive behaviors in male mice. In: Flannelly KJ, Blanchard RJ, Blanchard DC (eds) Biological perspectives on aggression. Liss, New York, pp 281–293

Robbins TW, Iversen SD (1973) A dissociation of the effects of *d*-amphetamine on locomotor activity and exploration in rats. Psychopharmacologia 28:155–164

Schultz LA, Kemble ED (1986) Prey-dependent effects of fluprazine hydrochloride on predatory aggression in Northern Grasshopper mice *(Onychomys leucogaster)* and rats *(Rattus norvegicus)*. Aggress Behav 12:267–275

Treit D (1985) Animal models for the study of anti-anxiety agents: a review. Neurosci Biobehav Rev 9:203–222