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Antianxiety and Behavioral Suppressant Actions of the Novel 5-HT_{1A} Receptor Agonist, Flesinoxan

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RODGERS, R. J., J. C. COLE AND A. DAVIES. *Antianxiety and behavioral suppressant actions of the novel 5-HT_{1A} receptor agonist, flesinoxan.* PHARMACOL BIOCHEM BEHAV **48**(4) 959–963, 1994.—Flesinoxan is a potent and selective 5-HT_{1A} receptor agonist. In this study, the effects of this compound on behavior in the murine elevated plus-maze have been assessed using a recently developed ethological scoring method. Results show that, at low doses (0.1–0.5 mg/kg), flesinoxan inhibited risk assessment behaviors (stretched attend postures and closed arm returns) indicative of a reduction in anxiety. These effects were maintained at a higher dose of 1.0 mg/kg, which also increased percent open entries and time spent on the central platform and open arms. However, this more convincing anxiolytic profile was associated with significant reductions in total arm entries and rearing, suggesting a combination of anxiolysis and behavioral suppression at high doses. The plus-maze profile observed with flesinoxan is very similar to that previously reported for 8-OH-DPAT in the same test but, despite superficial similarities, can be distinguished from that seen with buspirone. Data are discussed in relation to behavioral similarities and differences between 5-HT_{1A} receptor agonists, and the advantages of a more detailed approach to the analysis of plus-maze behavior.

Elevated plus-maze Ethological analysis Flesinoxan 5-HT_{1A} receptors Anxiety
Behavioral suppression Mice

FLESINOXAN, a phenylpiperazine derivative, has high affinity for 5-HT_{1A} receptors and much greater selectivity for these sites than either buspirone or ipsapirone. In binding assays, this novel compound displays 8-OH-DPAT-like affinity for 5-HT_{1A} receptors (K_i values of 1.7 nM vs. 2.8 nM, respectively), shows much lower (100–300 times) affinity for D_2, 5-HT_{1D}, and alpha$_1$ receptors, and has negligible affinity for other receptor populations (19,24). In brain, flesinoxan binding closely parallels that of 8-OH-DPAT, with highest concentrations found in limbic areas including CA1 and CA2 regions of the hippocampus, dentate gyrus, and lateral septum (19). Functionally, flesinoxan appears to act as a full 5-HT_{1A} receptor agonist, decreasing 5-HT turnover in brain, lowering body temperature, producing lower lip retraction, and showing complete crossgeneralization with 8-OH-DPAT in drug discrimination studies (12,19,25).

Despite this profile, our present knowledge of the behavioral pharmacology of flesinoxan is limited. It has been reported to reduce aggression in several animal models, but these effects are probably secondary to other behavioral changes (10,11,18). Antidepressant potential has been suggested by the compound's activity in the Porsolt test (22), its ability to downregulate beta-adrenoceptors (19) and its protective effect against long-lasting behavioral deficits induced by a single stress experience (21). However, in view of the clinical profile of other 5-HT_{1A} receptor agonists (20), it is unsurprising that most behavioral work on flesinoxan has focussed on its actions in animal models of anxiety. Anxiolytic-like effects have been reported in the pigeon conflict procedure (2), the murine stress hyperthermia test (26), and in both separation-induced (23) and foot shock-induced (17) ultrasonic vocalization tests in rats. Against this profile, the compound is purportedly inactive in a primate conflict procedure (7), as well as the four-plate and light/dark exploration tests in mice (19). This inconsistent pattern of results is puzzling in that it cannot readily be attributed to dose range, species, or general type of behavioral model used.

In the present study, our primary objective was to assess the effects of flesinoxan in a widely used and ecologically valid animal model of anxiety, the elevated plus-maze. Although

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this test has yielded inconsistent effects with 5-HT_{1A} receptor ligands (8,15), it has recently been reported that this sensitivity and reliability may be improved by adopting a more ethological approach to behavioral scoring (5,6,13,14,16). In particular, it has been shown that risk assessment behaviors (including head dipping, stretched attend postures, and closed-arm returns) are very sensitive both to anxiolytic and anxiogenic manipulations. For example, Cole and Rodgers (6) report low-dose anxiolytic-like (i.e., inhibitory) effects of acute buspirone on risk assessment. These effects were maintained across the dose range and were accompanied by a more general behavioral suppression only at high doses. Profile comparisons with the neuroleptic, haloperidol, suggested that the high dose behavioral suppressant effects of buspirone may be attributable to its D_2 antagonist properties. Thus, a secondary objective of the present study was to utilize the greater receptor selectivity of flesinoxan to further assess the validity of this proposal.

METHOD

Subjects

Subjects were 12–15 week old male DBA/2 mice (Biomedical Services, Leeds University), group housed (n = 10) for at least 4 weeks prior to testing (cage size: 45 × 28 × 13 cm). They were maintained under a 12D : 12L reversed light cycle (lights on: 1900 h) in a temperature-controlled environment (21 ± 1°C). Food and drinking water were freely available except during the brief test sessions. All mice were experimentally naive.

Drugs

Flesinoxan hydrochloride (formerly DU 29373: Solvay-Duphar, Weesp), (+)-N-[2-[4-(2,3-dihydro-2-hydroxymethyl-1,4-benzodioxan-5-yl)-1-piperazinyl]-ethyl]-4-fluorobenzamide HCl, was dissolved in physiological saline and administered IP (10 ml/kg) 20 min before testing. Doses cited refer to the salt.

Apparatus and Procedure

The elevated plus-maze was a modification of that validated for NIH Swiss mice by Lister (9). It comprised two open (30 × 5 × 0.25 cm) and two enclosed (30 × 5 × 15 cm) arms which extended from a common central platform (5 × 5 cm) which was elevated, by a single central support, to a height of 45 cm above floor level. The maze floor was made

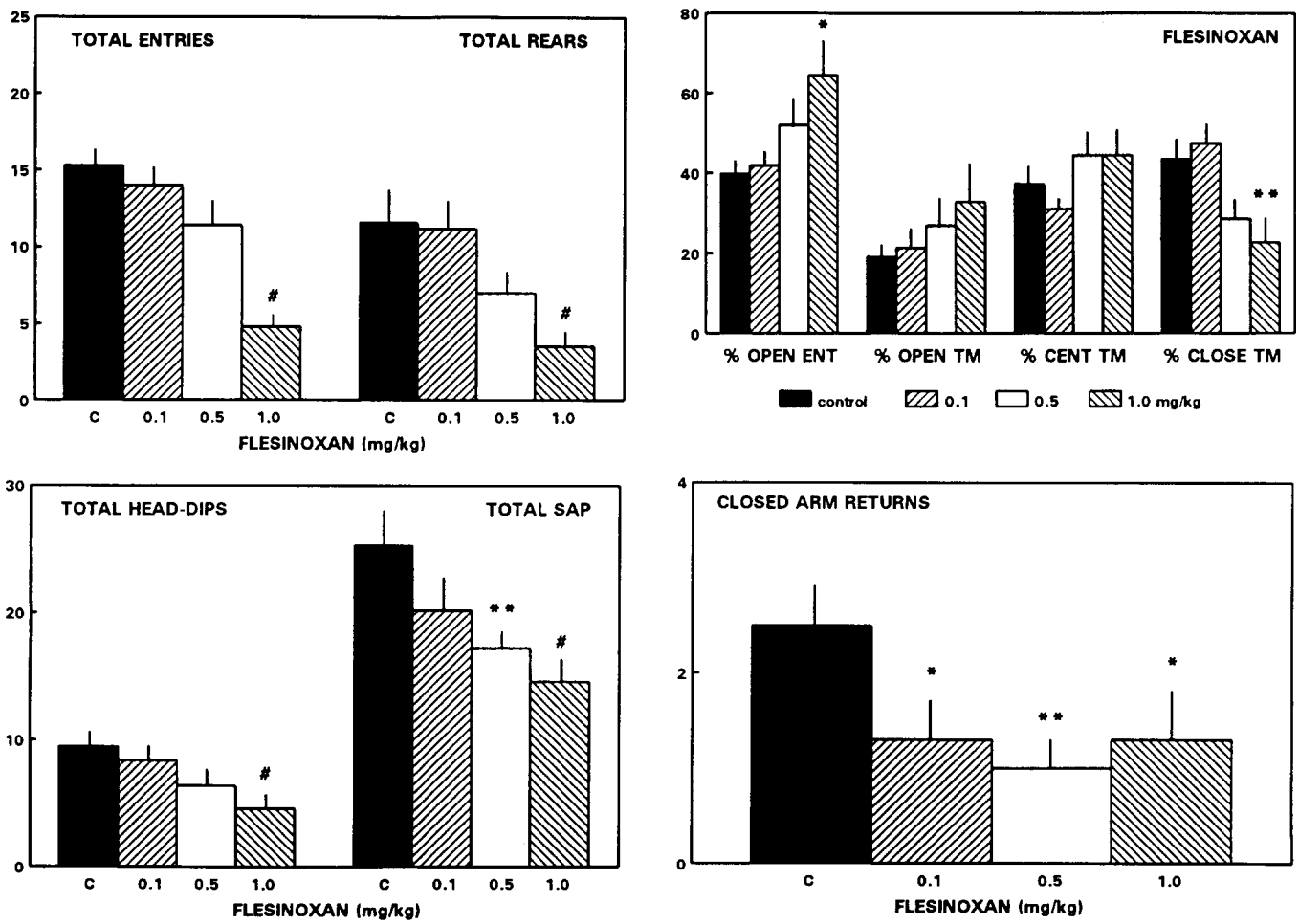

FIG. 1. Effects of flesinoxan (0.1–1.0 mg/kg, IP) on behaviors displayed by male DBA/2 mice in the elevated plus-maze. Data are expressed as mean values (±SEM). See text for full details, and Fig. 1 for complementary data. *$p < 0.05$, **$p < 0.025$, #$p < 0.005$ vs. saline control (ANOVA).

from black Plexiglas and the walls from clear Plexiglas. As previously reported (5,6,13,14,16), a slight raised edge on the open arms (0.25 cm) provided additional grip for the animals while open arm activity was further encouraged by testing under dim red light (2 × 60 W).

All testing was conducted during the dark phase of the light cycle and, to facilitate habituation, animals were transported to the laboratory and left undisturbed for at least 1 h prior to testing. Subjects were randomly allocated to treatment conditions (n = 10) and tested in counterbalanced order: saline control, 0.1, 0.5, or 1.0 mg/kg flesinoxan HCl. Twenty minutes following treatment, subjects were individually placed on the central platform of the maze facing an open arm. A standard 5-min test duration was employed, with all sessions recorded on videotape by an overhead camera linked to a monitor and VCR in an adjacent laboratory.

Behaviors, scored off videotape by a trained observer who remained blind to treatment condition, comprised both traditional and novel parameters (5,6,13,14,16).

Traditional spatiotemporal measures. Number of open and closed arm entries (arm entry = all paws into an arm), and time spent in various sections of the maze (including the central platform). Derived measures were the number of open arm entries expressed as a percentage of total arm entries (percent open entries), and time spent in different parts of the maze expressed as percentages of session duration (percent time open, percent time center, percent time closed).

Ethologically derived measures. Entry latency (time taken at start of session to move into a maze arm), nonexploratory behavior (combined duration of nonexploratory elements, immobility and grooming), closed arm returns (exiting a closed arm with only two paws, and returning/doubling back into the same arm), head dipping (HD; exploratory movement of head/shoulders directed over the sides of the maze), and stretched attend postures (SAP; an exploratory posture in which the mouse stretches forward and retracts to original position without actually locomoting forward). Head dipping and stretched attend postures were further differentiated as protected (i.e., occurring in/from the relative security of closed arms/central platform) or unprotected (i.e., occurring on or from the open arms). For these measures, data are expressed both as totals and as percent protected (protected/total × 100; percent pHD and percent pSAP) values.

Statistics

Data were analyzed by single-factor (treatment) or two-factor (treatment; location; repeated measures on second-factor) analyses of variance (ANOVA). Where indicated, either by significant main effects/interactions or F-values approaching significance, further comparisons were performed using Dunnett's t-statistic.

RESULTS

Data are summarized in Fig. 1 and Table 1.

ANOVA revealed significant main effects for flesinoxan on total arm entries, $F3, 36 = 12.10, p < 0.01$, open entries, $F3, 36 = 4.42, p < 0.025$, closed entries, $F3, 36 = 8.86, p < 0.01$, and total rearing, $F3, 36 = 5.55, p < 0.01$. Follow-up analyses revealed significant reductions in these behaviors at the highest dose tested ($p < 0.005$; Fig. 1).

Flesinoxan also dose dependently increased percent open arm entries, $F3, 36 = 3.49, p < 0.025$, reduced percent time spent in the enclosed arms of the maze, $F3, 36 = 4.88, p < 0.01$, and, reciprocally, increased percent time on the open arms and central platform (Fig. 1). Again, these effects were apparent at the top dose only ($p < 0.05$). On the percent time measure, mice displayed a distinct preference for different sections of the maze, $F2, 72 = 3.15, p < 0.05$. Saline controls spent equal amounts of time on the centre platform and closed arms and both these areas were strongly preferred over the open arms ($p < 0.02$). This pattern was significantly altered by flesinoxan, $F6, 72 = 2.40, p < 0.05$, such that, at 0.5 and 1.0 mg/kg doses, mice showed equal preference for open and closed arms, and spent more time on the central platform than in closed arms ($p < 0.05$).

Of the new measures recorded, flesinoxan failed to significantly influence either entry latency, $F3, 36 = 0.66$, NS, or nonexploratory behavior, $F3, 36 = 0.86$, NS (Table 1). However, significant treatment effects (Fig. 1) were found for head dipping, $F3, 36 = 4.5, p < 0.05$, and stretched attend postures, $F3, 36 = 4.73, p < 0.01$, while the F-value for closed arm returns just failed to reach statistical significance, $F3, 36 = 2.81$, NS; F(crit) = 2.84]. Follow-up tests confirmed that whereas reductions in head dipping were observed only at 1.0 mg/kg ($p < 0.005$), stretched attend postures were reduced at both 0.5 mg/kg ($p < 0.025$) and 1.0 mg/kg ($p < 0.005$),

TABLE 1

EFFECT OF FLESINOXAN (0.1-1.0 mg/kg, IP) ON BEHAVIORS DISPLAYED BY MALE DBA/2 MICE IN THE ELEVATED PLUS-MAZE TEST

Behavior	Flesinoxan (mg/kg)			
	Saline	0.1	0.5	1.0
Open entries	6.1 ± 0.6	5.6 ± 0.7	5.2 ± 0.7	3.1 ± 0.6*
Closed entries	9.2 ± 0.7	8.4 ± 1.5	6.2 ± 1.2	2.2 ± 0.7*
%pDips	49.6 ± 7.9	44.9 ± 9.1	29.1 ± 8.6	44.3 ± 14.4
%pSAP	59.2 ± 4.5	51.8 ± 5.0	46.0 ± 7.8	60.6 ± 10.3
Entry latency	6.1 ± 2.2	5.4 ± 2.8	12.5 ± 3.1	10.1 ± 6.8
Nonexploratory behavior (s)	17.1 ± 5.7	15.9 ± 4.3	8.5 ± 3.0	12.7 ± 2.9

Data are expressed as mean values (±SEM). %pDips and %pSAP refer to protected head-dipping and stretch attend postures expressed as a percentage of total behavioral frequencies. See text for full details, and Fig. 1 for complementary data.

*$p < 0.005$ vs. saline control (ANOVA).

while closed arms returns were inhibited across the entire dose range ($p < 0.05$–$p < 0.025$). No significant effects (Table 1) were found for percent protected head dipping, $F3, 36 = 0.74$, NS, or percent protected stretched attend postures, $F3, 36 = 0.87$, NS.

DISCUSSION

Risk assessment, a concept derived from studies on antipredator defense in rats, refers to a cluster of behavioral acts and postures that appear to serve an information-gathering function in potentially dangerous situations (3). Such behaviors, performed from relatively safe locations, comprise head outs and scanning of the danger area together with other cautious investigative behaviors such as flat-back approach and stretched attend postures. In the murine elevated plus-mase, risk assessment comprises head dipping, stretched attend postures, and closed arm returns, behaviors that occur with high probability at the junctions between closed arms, central platform, and open arms (5,6,13,14,16). Recent research, both on antipredator defense in rats [for review: (4)] and plus-maze behavior in mice [e.g., (5,6, 13,14,16)], has confirmed the sensitivity of such measures to behavioral and pharmacological treatments designed to manipulate anxiety levels.

The inclusion of risk assessment measures in the scoring of plus-maze behavior has also revealed that changes in anxiety can be detected even in the absence of significant treatment effects on the traditional indices of anxiety in this test. For example, the prototypical 5-HT_{1A} agonist, 8-OH-DPAT, reduces stretched attend postures at doses that fail to influence either open arm measures (percent entries, percent time) or general activity (16). Similar behavioral effects have been seen with acute administration of low doses of buspirone (6). Although higher doses of buspirone (acute or chronic) reduced risk assessment and increased open arm activity, concomitant behavioral suppression rendered data interpretation somewhat equivocal. However, on the basis of profile comparisons with the neuroleptic, haloperidol, it has been suggested that the anxiolytic-like effects of buspirone are a function of its 5-HT_{1A} agonist action while higher dose behavioral suppression may relate to additional D_2 antagonist activity (6).

The results of the present study show that the full 5-HT_{1A} receptor agonist, flesinoxan, reduced risk assessment behaviors (closed arm returns and total stretched attend postures) at doses (0.1–0.5 mg/kg) that did not significantly alter measures of general activity/exploration or traditional indices of anxiety. As risk assessment in the plus-maze is consistently inhibited by anxiolytic agents yet enhanced by anxiogenic treatments (5,6,13,14,16), these data agree well with reports concerning anxiolytic-like effects of flesinoxan in pigeons and rodents (2,17,23,26). Furthermore, as for 8-OH-DPAT (16) and buspirone (6), higher doses of flesinoxan (1.0 mg/kg) not only inhibited risk assessment and produced anxiolytic-like effects on percent open arm entries and time spent on different maze sections, but also resulted in a more general suppression of behavior. In view of the greater receptor selectivity of flesinoxan, this latter finding would appear to be at variance with the view that the behavioral suppression induced by high doses of buspirone is due to its D_2 antagonist actions (6). However, the advantage of an ethological approach is that it permits more detailed profile comparisons than are possible using total entry and total rearing scores alone.

In this context, the highest dose of flesinoxan tested (1.0 mg/kg) suppressed total arm entries (both open and closed entries) and total rearing, a pattern very similar to that found with high doses of 8-OH-DPAT in the same test (16). In contrast, the reduction in total entries previously reported for buspirone (2.5–5.0 mg/kg) was almost completely attributable to a reduction in closed arm entries (6). Furthermore, while the inhibition of total arm entries at high doses of buspirone was associated with marked increases in entry latency and nonexploratory behavior [i.e., immobility (6)], no such changes were apparent with flesinoxan at a dose (1 mg/kg) which reduced total entries to a comparable degree. These profile comparisons combine to suggest that different mechanisms may be responsible for the behavioral suppression observed with higher doses of flesinoxan and buspirone. A similar conclusion has been reached in a recent, detailed examination of the effects of 5-HT_{1A} receptor agonists on motor activity in rats. Ahlenius and colleagues (1) have shown that while motor activity is consistently reduced by 5-HT_{1A} receptor agonists, the precise nature of change is compound specific. Thus, flesinoxan and 8-OH-DPAT reduce the rate of activity (locomotion and rearing) and increase the proportion of forward locomotion, i.e., treated animals still move but more slowly. In contrast, the activity reduction induced with buspirone is associated with periods of immobility. The authors conclude that a) the effects seen with buspirone are much more similar to those seen with the D_2 receptor antagonists, haloperidol or raclopride, than those seen with 8-OH-DPAT or flesinoxan, and b) in all probability, these differences are due to the D_2 blocking effects of buspirone. Present data, along with plus-maze profiles already obtained for 8-OH-DPAT (16), buspirone (6), and haloperidol (6), would not be inconsistent with these conclusions.

In summary, present results show that flesinoxan reduces risk assessment in the murine elevated plus-maze, a finding that agrees well with the anxiolytic effects of other 5-HT_{1A} receptor agonists in this test. At higher doses, a more profound anxiety reduction is accompanied by behavioral suppression. Despite a superficial similarity to high dose buspirone effects (i.e., inhibition of total entries), more detailed behavioral comparisons suggest that different mechanisms are involved in the behavioral suppressant effects of these compounds.

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