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Ethopharmacological Analysis of the Effects of Putative 'Anxiogenic' Agents in the Mouse Elevated Plus-Maze

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RODGERS, R. J., J. C. COLE, K. ABOUALFA AND L. H. STEPHENSON. *Ethopharmacological analysis of the effects of putative 'anxiogenic' agents in the mouse elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **52**(4) 805–813, 1995.—The literature on the effects of anxiety-provoking agents in humans and animals is replete with inconsistent and contradictory findings as well as data that may have alternate explanations. To further our understanding in this area, ethological methods were used to assess in detail the effects of four putative anxiogenic agents in the murine elevated plus-maze test. Compounds studied were FG 7142 (1.25–10.0 mg/kg), pentylenetetrazol (PTZ; 1.875–30.0 mg/kg), isoproterenol (0.125–1.0 mg/kg), and sodium lactate (32.75–262.0 mg/kg). FG 7142 produced an anxiogenic-like profile at 10 mg/kg, an effect that could not be attributed to seizure activity or nonspecific behavioural suppression. PTZ exerted biphasic effects, with low doses (1.875–3.75 mg/kg) producing anxiolytic-like effects and high doses (20.0–30.0 mg/kg) anxiogenic-like effects. With the exception of the highest dose tested, which radically disrupted behavior, these effects of PTZ were also seen to be behaviorally specific. Although some minor behavioural changes were evident with sodium lactate and isoproterenol, neither compound altered anxiety-related measures under present test conditions. Data are discussed in relation to distinctions between anxiety and panic, and the nature of anxiety expressed in and detected by animal models.

Elevated plus-maze Ethological analysis Anxiogenic FG 7142 Pentylenetetrazol Sodium lactate Isoproterenol/ Mice

MANY agents have been reported to provoke anxiety- or panic-like reactions in human volunteers and/or patients, with some effects more robust than others. These substances include carbon dioxide, sodium lactate, isoproterenol, pentylenetetrazol (PTZ), caffeine, yohimbine, certain β-carbolines (e.g., FG 7142), *m*-chlorophenylpiperazine (mCPP), fenfluramine, cholecystokinin tetrapeptide (CCK-4), as well as withdrawal from chronic benzodiazepine or ethanol treatment (3,4,41). Most, if not all, of these treatments are also capable of inducing/enhancing anxiety in experimental animals. However, even for supposedly well-established anxiogenics, it is clear that some (e.g., FG 7142, PTZ) produce either inconsistent effects or effects that may have alternative interpretations, while others (e.g., sodium lactate, isoproterenol) have not been extensively tested in animals (26,36,47,58,60).

As a benzodiazepine receptor inverse agonist, the β-carboline, FG 7142, would be expected to produce behavioral effects opposite to those of diazepam. Correspondingly, the compound has been reported to have anxiogenic-like effects in humans (17), and in animal models including both traditional conflict procedures (11,56) and tests of unconditioned behavior such as the social interaction (21), separation-induced ultrasonic vocalization (40), and social competition (30) procedures. Although findings in the elevated plus-maze would generally support an anxiogenic action (13,15,20,34,44), many of these studies report concomitant reductions in overall activity, while yet others have actually failed to detect a convincing anxiogenic profile (e.g., 38,55,57). Negative results have also been obtained in shock-induced/startle-induced ultrasonic vocalization paradigms (16,32) and the light-dark exploration test (12). In addition, several authors have questioned the specificity of the compound's effects on punished responding in conflict tests (42,62). These and similar findings have led Thiebot and colleagues (58) to express doubt as to the specificity of FG 7142-induced anxiogenesis.

PTZ is a chemoconvulsant that acts via the picrotoxin site on the benzodiazepine/GABA receptor complex to reduce chloride influx (43). Like FG 7142, this compound has been reported to induce anxiety in humans (52) and to exert anxiogenic-like effects in animal models ranging from traditional operant conflict tasks (10,53,56) to tests of spontaneous behavior such as the social interaction (29), separation-induced

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ultrasound (7,40), light-dark exploration (14), social competition (30), novelty-induced thigmotaxis (54), and elevated plus-maze (6,13,29,33,37,64) paradigms. Despite these findings, and the elegant demonstration of generalization between a PTZ cue and the subjective aftereffects of predator exposure (23), active doses of PTZ are often seen to impair general activity. Furthermore, PTZ has not always been found to enhance anxiety. For example, the compound has been reported to have nonspecific effects on punished responding (27), no effects in the stretched attend posture (SAP) test (31), anxiolytic-like effects in the shock-induced ultrasonics and defensive burying tests (16,59), and to induce a conditioned place preference in rats (22). Furthermore, questions have inevitably been raised about the possible contribution of convulsant effects to its behavioral profile (2).

Sodium lactate and isoproterenol are the two most frequently used anxiety-provoking agents in human studies (3,4,41). However, to our knowledge, only one study has examined the effects of these agents in animal models of anxiety (28). In that report, neither compound was found to be effective in the rat social interaction model while, in the plus-maze test, only isoproterenol produced effects consistent with a mild increase in anxiety.

The aim of the present study was to assess in detail the behavioral effects of these four putative anxiogenic agents in the murine elevated plus-maze test. To enhance our ability to detect behavioral changes with (and between) the compounds, we employed an ethological version of the test. This modification to standard methodology includes the scoring of specific defensive behaviors in addition to the more usual spatiotemporal measures, and has recently proven very sensitive to increases (46,49) as well as decreases (8,9,50) in anxiety. The comprehensive behavioral profiles yielded by this technique also provide information invaluable to the question of the behavioral specificity of drug action.

GENERAL METHOD

Animals

Subjects were adult male DBA/2 mice (Biomedical services, University of Leeds), aged 12–15 weeks at the time of testing. They were housed in groups of 10 (cage size: 45 × 28 × 13 cm) and maintained under a 12-h reversed light cycle (lights off: 0700 h) in a temperature-controlled environment (21 ± 1°C) for at least 4 weeks prior to testing. Food and water were freely available except for the brief test sessions. Naive mice were used for each experiment.

Drugs

Drugs used were FG 7142 [Research Biochemicals Incorporated (RBI), USA], pentylenetetrazol (PTZ; Sigma, Dorset, UK), (±)isoproterenol hydrochloride (RBI), and sodium lactate (Sigma). FG 7142 was ultrasonically dispersed in saline to which two drops of Tween 80/10 ml had been added while sodium lactate was prepared from stock solution supplied by Sigma. PTZ and isoproterenol were dissolved in physiological saline. Control groups received injections of the appropriate vehicle. All compounds were prepared freshly on test days and administered IP in a volume of 10 ml/kg 30 min prior to testing.

Apparatus

The plus-maze, based on that designed and validated by Lister (34), was elevated to a height of 45 cm above floor level and comprised two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 15 cm) that extended from a common central platform (5 × 5 cm). The maze floor was made from black Plexiglas while, to aid visibility of the animals and to avoid dark recesses in the apparatus, the side- and end-walls of the enclosed arms were made from clear Plexiglas. Grip on the open arms was facilitated by a raised edge (0.25 cm) around their perimeter while open arm activity was further encouraged by testing under red light (3 × 60 W).

Procedure

All testing was conducted during the dark phase of the light cycle (between 1000 and 1400 h) and, to facilitate adaptation, animals were transported to the laboratory at least 1 h prior to the onset of testing. For each study, mice were randomly allocated to treatment conditions (n = 9–15) and tested in counterbalanced order. Testing commenced by placing a subject on the central platform of the maze facing an open arm. A 5-min test duration was employed and, between subjects, the maze was cleaned with damp and dry cloths. All test sessions were recorded by an overhead videocamera that was linked to a monitor and VCR in an adjacent laboratory. Tapes were subsequently scored by a highly trained observer who remained blind to treatment condition until all analyses were completed.

Behavioral Analysis

Videotapes were scored for several behavioral measures in addition to the standard spatiotemporal measures. Standard parameters comprised number of open, closed and total arm entries (arm entry defined as all four paws into an arm), and time spent on different sections of the maze (including the central platform). The spatial and temporal distribution of behavior was additionally calculated as percent total for both frequency (percent open entries) and duration (percent time spent on open, center, and closed sections) data.

The behavioral measures recorded relate to the defensive repertoire of the mouse (46,49), and included entry latency (time taken at start of session to move from the center platform into an arm) and nonexploratory behavior (the combined duration of immobility and grooming), as well as a number of risk assessment measures. The latter characterize the behavior of more cautious subjects and comprised head dipping (exploratory scanning over the sides of the maze), stretched attend postures (SAP; forward elongation of the head and shoulders followed by retraction to original position), and closed arm returns (exiting a closed arm with head and forepaws only and then retreating/doubling back into the same arm). In view of the importance of thigmotactic cues to behavior on the maze (61), head dipping and stretched attend postures were differentiated by location as either protected (i.e., occurring from the relative security of the closed arms) or unprotected (i.e., occurring on or from an open arm). Analogous to calculations for open arm entries/time, data for head dipping and SAP are presented both as totals and as percent protected values (%pDips, %pSAP; = protected/total × 100).

Statistical Analysis

Data were analyzed by single-factor (treatment) or two-factor (treatment and maze location, with repeated measures on location) analyses of variance (ANOVA). Where indicated by significant/near-significant F-values, further comparisons

were performed using the appropriate error variance terms from the ANOVA summary tables (i.e., Dunnett's *t*-statistic).

RESULTS

FG 7142

Data are summarized in Fig. 1 and Table 1. ANOVA indicated that FG 7142 had significant effects on open arm entries, $F(4, 45) = 4.29$, $p < 0.01$, and percent open entries, $F(4, 45) = 3.73$, $p < 0.01$, both reflecting reductions at 10.0 mg/kg ($p < 0.005$). As the compound did not affect closed entries, $F(4, 45) = 0.92$, NS, the trend towards a significant reduction in total entries, $F(4, 45) = 2.42$, NS; $Fcrit_{0.05} = 2.53$, is clearly accounted for by the reduction in entries onto the open arms. This was confirmed by follow-up tests that showed that total entries were significantly reduced at 10 mg/kg ($p < 0.01$). On the percent time measure, mice showed a clear preference for different maze locations, with closed arms preferred over the central platform, and both of these areas preferred over the open arms, $F(2, 90) = 219.3$, $p < 0.001$. Although this pattern was apparently unaffected by FG 7142, $F(8, 90) = 1.54$, NS, Fig. 1 clearly suggests trends towards reduced time on the open arms, $F(4, 45) = 2.03$, NS, and increased time in the enclosed arms, $F(4, 45) = 1.79$, NS. No such trends are apparent for time spent on the centre platform, $F(4, 45) = 0.13$, NS.

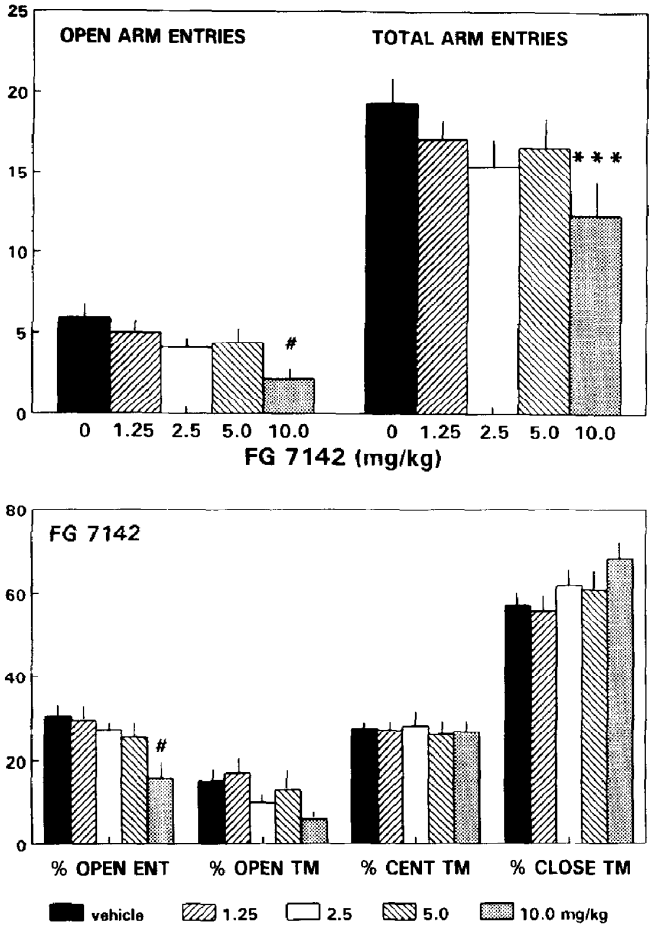

FIG. 1. Effects of FG 7142 (1.25–10.0 mg/kg) on the behavior (open and total arm entries; percent time spent on open, center, and enclosed parts of the maze) of male mice in the elevated plus-maze. Data are presented are mean values ± SEM. See also Table 1. ***$p < 0.01$, #$p < 0.005$ vs. control.

In addition to spatiotemporal measures, specific behavioural parameters were sensitive to the effects of FG 7142. These changes were most clearly seen in closed arm returns, $F(4, 45) = 2.95$, $p < 0.05$, and nonexploratory behavior, $F(4, 45) = 3.14$, $p < 0.025$, with increases evident at 5.0 mg/kg ($p < 0.005$) and at 2.5 and 10.0 mg/kg ($p < 0.05$ to $p < 0.025$), respectively. Although the *F*-value for rearing did not quite reach an acceptable level of significance, $F(4, 45) = 2.21$, NS; $Fcrit_{0.05} = 2.53$, the apparent reduction at 10 mg/kg was confirmed in follow-up tests ($p < 0.05$). FG 7142 did not affect entry latency, $F(4, 45) = 1.87$, NS, total head dipping, $F(4, 45) = 0.97$, NS, stretched attend postures, $F(4, 45) = 1.11$, NS, or the protected forms of the latter behaviors (%pDips–$F(4, 45) = 0.30$, NS; %pSAP–$F(4, 45) = 0.65$, NS.

Pentylenetetrazol

Pilot studies had suggested possible biphasic effects of PTZ in the plus-maze and, as such, more dose levels than normal were examined. Data are summarized in Figs. 2–4. Statistical analysis showed that PTZ affected almost all plus-maze measures taken, and confirmed that very different effects are produced by low (< 4 mg/kg) and high (> 15 mg/kg) doses of this compound.

PTZ significantly altered closed arm entries, $F(6, 65) = 12.94$, $p < 0.001$, open arm entries, $F(6, 65) = 10.40$, $p < 0.001$), total arm entries, $F(6, 65) = 17.12$, $p < 0.001$, and percent open entries, $F(6, 65) = 9.69$, $p < 0.001$. Closed arm entries were significantly reduced at 15 mg/kg ($p < 0.05$) and 30 mg/kg ($p < 0.005$), while total entries were reduced only at the highest dose tested ($p < 0.005$). In contrast, both open arm entries and percent open arm entries displayed a biphasic response to drug treatment with increases at low doses (1.875–3.75 mg/kg, $p < 0.05$ to $p < 0.025$), and decreases at high doses (20–30 mg/kg, $p < 0.05$ to $p < 0.005$). On the percent time measure, the rank order preference of closed arms > center platform > open arms, $F(2, 130) = 85.33$, $p < 0.001$, was significantly altered by drug treatment, $F(12, 130) = 2.93$, $p < 0.01$. Further analysis indicated that PTZ significantly altered both percent time spent on the open arms, $F(6, 65) = 6.79$, $p < 0.01$, and time spent on the center platform, $F(6, 65) = 3.44$, $p < 0.01$. A biphasic effect was apparent for time open, with an increase at the lowest dose tested ($p < 0.025$) and reductions at 20–30 mg/kg ($p < 0.05$). A substantial increase in time center was noted at the highest dose tested ($p < 0.005$).

PTZ also produced significant effects on specific plus-maze behaviors, with changes evident in entry latency, $F(6, 65) = 9.63$, $p < 0.001$, rearing, $F(6, 65) = 9.31$, $p < 0.001$, head dipping, $F(6, 65) = 6.49$, $p < 0.01$, stretched attend postures, $F(6, 65) = 16.76$, $p < 0.001$, %pSAP, $F(6, 65) = 6.11$, $p < 0.01$, closed arm returns, $F(6, 65) = 8.82$, $p < 0.001$, and nonexploratory behavior, $F(6, 65) = 52.60$, $p < 0.001$. Further analysis revealed biphasic effects of PTZ on head dipping (increased at 1.875 mg/kg, $p < 0.05$; reduced at 30 mg/kg, $p < 0.005$) and stretched attend postures (increased 1.875–7.5 mg/kg, $p < 0.005$; reduced at 30.0 mg/kg, $p < 0.005$). Closed arm returns were enhanced at 20 mg/kg (data not shown; control 1.3 ± 0.4 vs. PTZ20 3.8 ± 0.8, $p < 0.005$), while %pSAP and nonexploratory behavior were

TABLE 1

EFFECTS OF FG 7142 (1.25-10.0 mg/kg, IP) ON THE BEHAVIOR OF MALE MICE IN THE ELEVATED PLUS-MAZE

	FG 7142 (mg/kg)				
Behavior	Vehicle	1.25	2.5	5.0	10.0
Closed arm entries	13.5 ± 1.2	12.1 ± 1.0	11.3 ± 1.3	12.1 ± 1.2	10.2 ± 1.6
Closed arm returns	1.1 ± 0.3	1.7 ± 0.4	1.9 ± 0.3	3.3 ± 0.7*	2.2 ± 0.6
Total rears	11.3 ± 1.0	11.3 ± 1.3	8.0 ± 1.1	10.2 ± 2.0	6.5 ± 2.0†
Entry latency (s)	7.2 ± 1.9	3.7 ± 1.0	7.8 ± 2.0	3.9 ± 1.3	9.3 ± 2.0
NEB (s)	24.7 ± 3.6	25.2 ± 5.6	57.8 ± 12.5†	44.4 ± 10.6	70.7 ± 22.6‡
Total head-dips	4.3 ± 0.5	3.6 ± 0.6	3.9 ± 0.5	4.2 ± 0.8	2.6 ± 1.0
% p Dips	72.2 ± 8.4	67.9 ± 9.9	70.0 ± 8.6	61.6 ± 11.7	78.0 ± 11.2
Total SAP	17.8 ± 0.9	15.9 ± 0.9	15.7 ± 1.6	16.5 ± 2.1	13.3 ± 2.2
% p SAP	68.7 ± 5.5	69.9 ± 6.3	76.7 ± 4.7	78.3 ± 9.5	81.2 ± 9.2

Data are presented as mean values (± SEM). % p Dips—percent protected head-dipping; % p SAP—percent protected stretched attend postures; NEB = nonexploratory behavior. See also Fig. 1.
*$p < 0.005$ vs. vehicle; †$p < 0.05$; ‡$p < 0.025$.

significantly increased at 20-30 mg/kg ($p < 0.05$ tp $p < 0.005$). Only the protected form of head dipping (%pDips) remained unaffected by drug treatment, $F(6, 65) = 1.78$, NS.

Isoproterenol

Data and ANOVA statistics are summarized in Table 2. Mice showed a significant rank order preference for time spent in different parts of the maze, with closed arms > center platform > open arms, $F(2, 90) = 92.09$, $p < 0.001$. This profile was not affected by drug treatment, $F(8, 90) = 0.61$, NS. Indeed, with the exception of entry latency, which showed a significant decrease across the dose range tested (0.125-1.0 mg/kg; $p < 0.05$ to $p < 0.005$), isoproterenol was largely without effect on behavior in the elevated plus-maze. Several F-values closely approached significance ($F\text{crit}_{0.05} = 2.53$), including rearing (2.35), stretched attend postures (2.33), closed entries (2.24), and total entries (2.38). Further analysis indicated significant reductions in rearing and SAP at 1.0 mg/kg ($p < 0.05$), with increases in closed/total arm entries at the lowest dose tested that just failed to reach significance.

Sodium Lactate

Data and ANOVA statistics are summarized in Table 3. Mice were again seen to show a distinct rank order preference for time spent in different sections of the maze, with closed > center > open, $F(2, 88) = 46.79$, $p < 0.001$. Over the dose range tested, sodium lactate did not alter this basic profile, $F(8, 88) = 1.07$, NS. Indeed, the only measure to be significantly affected by drug treatment was rearing, $F(4, 44) = 2.73$, $p < 0.05$, with a reduction in this measure evident at the lowest dose tested ($p < 0.01$). Although a nonsiginficant F-value was obtained (Table 3), follow-up tests showed that the lowest dose of sodium lactate also increased %pDips ($p < 0.05$).

DISCUSSION

A review of the literature suggests that the effects of anxiety-provoking drugs in animals and humans can be highly variable. This conclusion is confirmed and extended in the present study of four putative anxiogenic agents in the murine plus-maze. Using wide dose ranges of each compound and in-depth behavioral analysis, our data confirm the anxiety-enhancing effects of the β-carboline, FG 7142, and demon-

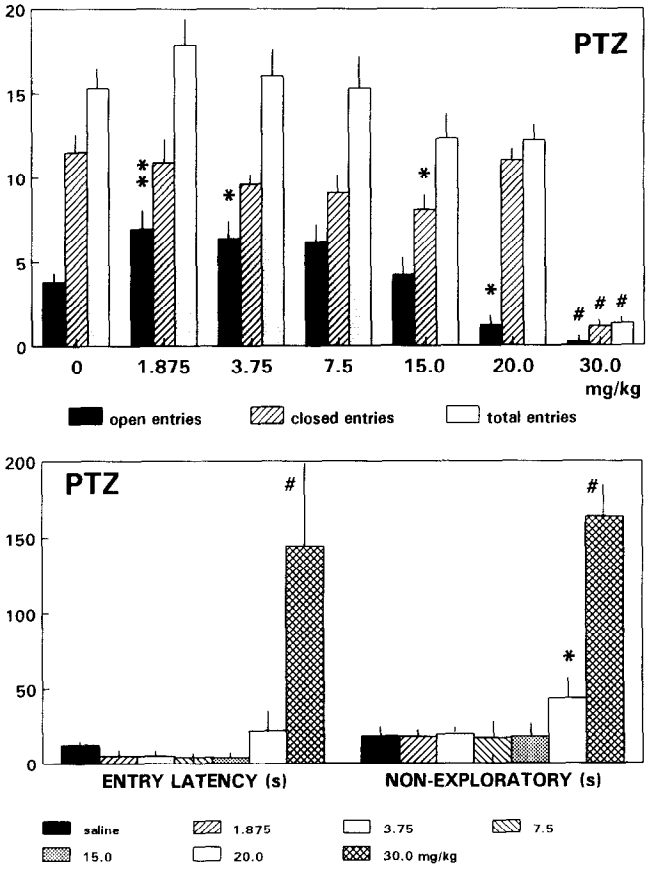

FIG. 2. Effects of PTZ (1.875-30.0 mg/kg) on the behavior (open/closed/total arm entries; entry latency and nonexploratory behavior) of male mice in the elevated plus-maze. Data presented are mean values ± SEM. See also Figs. 3 and 4. *$p < 0.05$; **$p < 0.025$; #$p < 0.005$ vs. control.

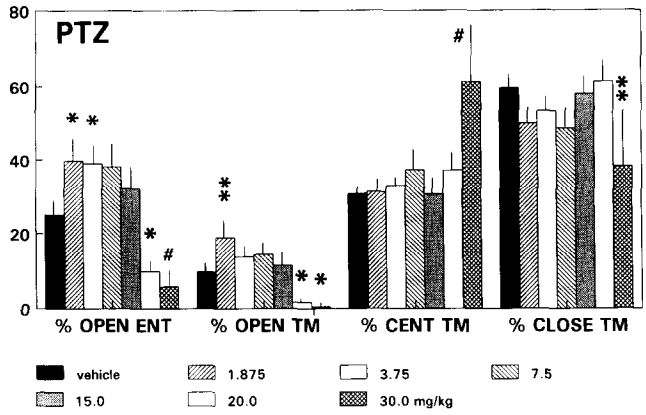

FIG. 3. Effects of PTZ (1.875–30.0 mg/kg) on the behavior (percent open entries; percent time spent in open, center, and enclosed parts of the maze) of male mice in the elevated plus-maze. Data presented are mean values ± SEM. See also Figs. 2 and 4. $*p < 0.05$; $**p < 0.025$; $\#p < 0.005$ vs. control.

strate an apparent biphasic effect on anxiety of the CNS stimulant/convulsant, PTZ. In contrast, our results also show that sodium lactate and isoproterenol are completely without effect on anxiety as assessed in this paradigm.

In agreement with earlier findings (13,15,20,34,44), FG 7142 produced a dose-dependent increase in anxiety that reached significance on key measures at the higher doses tested (5–10 mg/kg). It is important to note that these effects were seen in the absence of any overt seizure activity, which, in this mouse strain, requires considerably higher doses (51). A 5 mg/kg dose significantly elevated closed arm returns, a measure that has previously been shown to be very sensitive to anxiety-enhancing treatments [e.g., (46,49)]. At 10 mg/kg, open arm entries and percent open entries were both significantly reduced, a profile also consistent with an increased anxiety. This interpretation is supported by the clear (though nonsignificant) trends towards reduced time spent on the open arms and increases in protected stretched attend postures. A pattern of reduced percent open entries in the absence of a significant reduction in percent open arm time was also reported by Lister (35). Although the observed reduction in rearing and concomitant increase in nonexploratory behavior might be seen as evidence of behavioral nonspecificity, it is important to note that a) there was no change in closed arm entries, the measure widely considered to be the most valid index of general activity in this test (13,18,48); b) no significant changes were noted in a variety of other measures known to be sensitive to motor deficits [i.e., entry latency, head dipping, and stretched attend postures; e.g., (9,50)]; and c) nonpharmacological stressors (such as exposure to social defeat or the scent of an aggressive conspecific) have also been found to reduce rearing and increase nonexploratory behavior in this test and, as such, these particular behavioral changes cannot be taken as evidence of a nonspecific drug effect (47).

PTZ was tested up to a maximum dose of 30 mg/kg without evidence of overt seizures. This would be generally consistent with other studies that report that 30 mg/kg is below seizure threshold for PTZ in various mouse strains (5,19,25, 39,45). Our plus-maze data provide evidence for a biphasic action of PTZ in the murine elevated plus-maze test, a profile that has not previously been reported. Low doses (1.875–3.75 mg/kg) were found to have anxiolytic-like effects. Thus, in the absence of changes in general activity (total entries, closed entries, rearing), the compound was seen to produce benzodiazepine-like increases in open entries, percent open entries, percent open time, and head dipping. The latter effect, coupled with increase in total SAP, further supports a stimulation of open arm exploration at these low doses. This apparent reduction in anxiety may be related to the possibly pleasurable effects of mild arousal, an action that has been suggested as responsible for the drug's ability to induce a place preference (22) and to enhance learning (63).

The increase in total SAP seen with low doses of PTZ deserves particular comment in that previous research from this laboratory might suggest that total SAP should decrease with anxiolytics [e.g., (8)] and increase with anxiogenics [e.g., (49)]. However, that this is not always the case is confirmed by the observation that the benzodiazepine receptor partial agonist, bretazenil, produces anxiolytic-like effects on a range of plus-maze measures in the absence of a change in total SAP (8). Nevertheless, the compound does reduce the proportion of such postures displayed from protected areas of the maze, a profile consistent with anxiety reduction. In this context, close examination of Fig. 4 reveals that, despite enhancing total SAP, low doses of PTZ also reduce percent protected SAP, albeit nonsignificantly. This pattern is clearly indicative of a shift in risk assessment from protected to unprotected

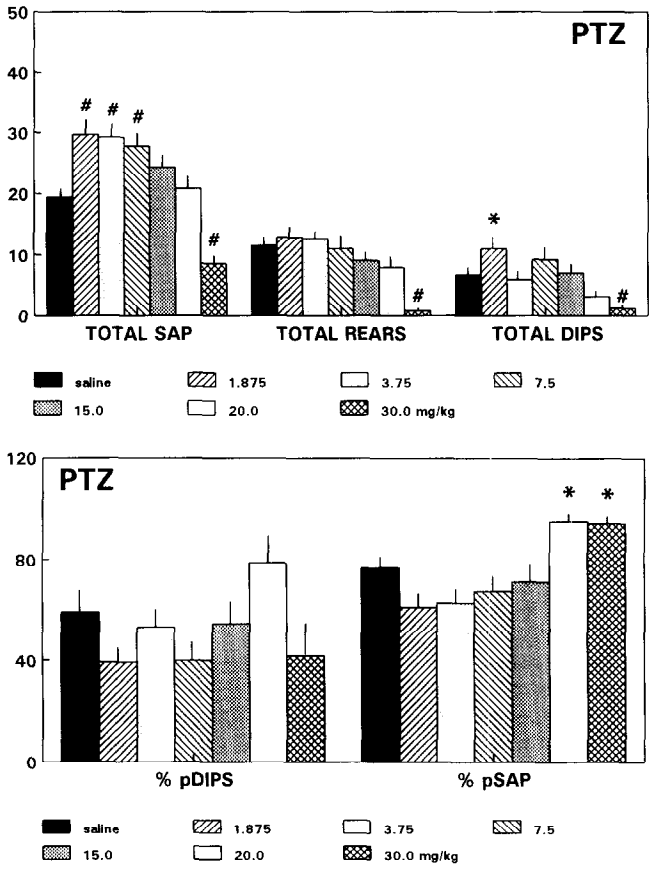

FIG. 4. Effects of PTZ (1.875–30.0 mg/kg) on the behavior (SAP; rears; head dips; %pDips; %pSAP) of male mice in the elevated plus-maze. Data presented are mean values ± SEM. See also Figs. 2 and 3. SAP = stretched attend postures; %p = percent protected (see text for full explanation). $*p < 0.05$; $\#p < 0.005$.

TABLE 2

EFFECTS OF ISOPROTERENOL (0.125–1.0 mg/kg, IP) ON THE BEHAVIOR OF MALE MICE IN THE ELEVATED PLUS MAZE

	Isoproterenol (mg/kg)					
Behavior	Saline	0.125	0.25	0.5	1.0	$F_{(4,45)}$
Total entries	14.4 ± 1.3	18.0 ± 3.2	14.9 ± 1.4	10.8 ± 1.1	11.5 ± 1.5	2.38, NS
Total rears	8.1 ± 0.8	8.4 ± 1.4	4.7 ± 1.5	4.0 ± 1.3	4.1 ± 0.9	2.35, NS
Open entries	6.4 ± 0.9	5.8 ± 1.2	6.0 ± 1.0	3.9 ± 0.6	4.2 ± 0.8	1.54, NS
Closed entries	8.0 ± 1.2	12.2 ± 2.4	8.9 ± 0.9	6.9 ± 0.9	7.3 ± 1.2	2.24, NS
% Open entries	45.4 ± 5.8	32.4 ± 5.3	37.8 ± 5.6	35.4 ± 4.6	35.8 ± 6.5	0.78, NS
% Open time	15.6 ± 1.9	16.4 ± 3.2	16.4 ± 3.5	14.3 ± 5.1	11.4 ± 2.6	0.38, NS
% Closed time	55.9 ± 4.1	53.9 ± 3.2	59.2 ± 5.0	58.6 ± 4.8	53.0 ± 5.0	0.38, NS
% Centre time	28.5 ± 4.0	29.7 ± 2.6	24.4 ± 2.7	27.1 ± 5.3	35.6 ± 4.7	1.07, NS
% Total head-dips	4.8 ± 0.9	5.7 ± 1.3	3.7 ± 0.8	6.9 ± 1.2	5.0 ± 1.5	1.04, NS
% p Dips	54.5 ± 7.2	57.3 ± 10.7	43.6 ± 8.2	53.9 ± 10.7	73.2 ± 10.7	1.23, NS
Total SAP	16.3 ± 1.4	14.3 ± 1.9	11.8 ± 0.9	13.5 ± 1.2	11.0 ± 1.1	2.33, NS
% p SAP	62.5 ± 4.8	65.0 ± 5.8	64.6 ± 7.0	72.5 ± 7.4	76.6 ± 7.2	0.85, NS
Closed arm returns	1.1 ± 0.4	1.6 ± 0.7	1.5 ± 0.5	0.9 ± 0.4	0.5 ± 0.3	1.00, NS
Entry latency(s)	18.6 ± 6.3	2.5 ± 0.9*	1.8 ± 0.5*	6.1 ± 2.2†	7.6 ± 3.5‡	3.88, $p < 0.01$
NEB(s)	27.7 ± 5.7	23.9 ± 6.6	25.7 ± 5.6	40.3 ± 8.4	26.7 ± 5.1	1.06, NS

Data are presented as mean values (±SEM). % p Dips—percent protected head-dipping; % p SAP—percent protected stretched attend postures; NEB = nonexploratory behavior.

*$p < 0.005$ vs saline; †$p < 0.025$; ‡$p < 0.05$.

areas of the maze, i.e., anxiolysis. In contrast, higher doses of PTZ, which either have no effect upon or actually reduce SAP, significantly increase percent protected SAP measures (see below). A similar point can also be made with reference to Table 1, which shows that the β-carboline anxiogenic, FG 7142 (2.5–10.0 mg/kg), while not significantly altering total SAP, increased percent protected SAP by approximately 12%. It is, therefore, apparent that total SAP cannot be used as an index of anxiety in isolation from other factors. As indicated, the distribution of the behavior on the maze is very important to interpretation as are simultaneous changes to other aspects of the behavioral repertoire. Thus, as for other behaviors, total SAP can be/is affected by treatments impacting arousal levels. It is, therefore, imperative to consider the wider behavioral context within which alterations in this measure are observed.

TABLE 3

EFFECTS OF SODIUM LACTATE (32.75–262.0 mg/kg, IP) ON THE BEHAVIOR OF MALE MICE IN THE ELEVATED PLUS-MAZE

	Sodium lactate (mg/kg)					
Behavior	Saline	32.75	65.5	131.0	262.0	$F_{(4,45)}$
Total entries	16.3 ± 1.4	11.2 ± 1.9	15.8 ± 1.8	12.4 ± 1.9	11.9 ± 1.5	1.89, NS
Total rears	8.9 ± 1.6	3.7 ± 0.9*	9.1 ± 1.2	6.7 ± 1.7	5.8 ± 1.2	2.73, $p < 0.05$
Open entries	5.2 ± 1.2	3.0 ± 0.7	5.6 ± 1.2	4.5 ± 0.8	3.6 ± 0.7	1.36, NS
Closed entries	11.1 ± 0.9	8.2 ± 1.7	10.2 ± 1.3	7.9 ± 1.3	8.3 ± 1.2	1.13, NS
% Open entries	29.7 ± 4.9	39.9 ± 10.9	33.8 ± 6.4	35.8 ± 3.6	32.0 ± 4.7	0.35, NS
% Open time	14.7 ± 3.1	9.4 ± 3.0	16.5 ± 3.3	16.6 ± 2.1	13.9 ± 3.5	0.95, NS
% Closed time	57.3 ± 3.2	45.8 ± 8.6	52.7 ± 4.1	45.7 ± 4.9	50.6 ± 6.6	0.71, NS
% Centre time	28.0 ± 2.2	44.8 ± 8.8	30.8 ± 2.4	37.7 ± 5.4	35.5 ± 3.9	1.56, NS
Total head-dips	5.1 ± 0.8	4.1 ± 0.8	5.7 ± 1.2	4.9 ± 1.0	3.8 ± 0.6	0.72, NS
% p Dips	36.5 ± 10.6	72.2 ± 10.8*	49.3 ± 12.0	36.1 ± 8.8	51.7 ± 12.8	1.82, NS
Total SAP	18.4 ± 2.2	12.9 ± 1.8	16.7 ± 1.5	13.8 ± 2.0	17.4 ± 1.8	1.66, NS
% p SAP	71.8 ± 5.3	71.6 ± 9.8	71.3 ± 6.9	73.8 ± 4.3	63.6 ± 8.9	0.29, NS
Closed arm returns	2.3 ± 0.8	1.4 ± 0.6	2.1 ± 0.7	1.0 ± 0.4	1.2 ± 0.5	0.92, NS
Entry latency(s)	3.7 ± 1.3	9.2 ± 4.7	3.2 ± 1.6	2.8 ± 1.2	4.8 ± 3.3	0.86, NS
NEB(s)	26.6 ± 8.2	31.6 ± 7.2	23.7 ± 4.8	25.4 ± 6.6	26.0 ± 4.2	0.21, NS

Data are presented as mean values (±SEM). % p Dips—percent protected head-dipping; % p SAP—percent protected stretched attend postures; NEB = nonexploratory behavior.

*$p < 0.01$ vs. saline.

Intermediate doses of PTZ (7.5–15 mg/kg) were largely inactive under present test conditions but, at higher doses, clear evidence of anxiety enhancement was seen. Although this finding is consistent with previous research (6,13,29, 33,37,64), ethological analysis revealed an important difference in the effects of the top two doses of the compound. PTZ (20 mg/kg) reduced open entries, percent open entries, and percent open time, while increasing percent protected SAP and closed arm returns. Although nonexploratory behavior was increased at this dose, the lack of drug effect on total entries, closed entries, head dipping, and rearing suggests that the change in nonexploratory behavior was due to a drug-induced increase in freezing and/or grooming rather than motoric disruption. Although generally similar effects on these anxiety-related parameters were seen at the highest dose tested (30 mg/kg), these were accompanied by a profound behavioral suppression: major reductions were evident in total entries, closed entries, rearing, head dipping, and stretched attend postures with corresponding increases in entry latency and nonexploratory behavior. Indeed, as the latter scores were very similar to one another (circa 150s), animals treated with 30 mg/kg PTZ did not actually move from the center platform until approximately half-way through the 5-min test session. This interpretation is fully confirmed by the substantial increase in time spent on the center platfrom at this dose level. Together, these data allow the conclusion that PTZ induces anxiogenesis at 20 mg/kg but behavioral disruption at higher doses. Although no overt signs of seizures were apparent at 30 mg/kg, the virtual elimination of active behavior might indicate the existence of a preconvulsant state.

The behavioral profiles obtained with FG 7142 and PTZ in the present study are consistent with the anxiogenic-like effects previously seen in the same test following treatment with compounds such as mCPP and TFMPP (49) or exposure to social stressors (46). Together, these findings clearly confirm the sensitivity of the murine elevated plus-maze test to treatments that promote anxiety. It is, therefore, very revealing that, over the dose ranges tested, neither sodium lactate nor isoproterenol produced any signs of anxiety enhancement. This result is in general agreement with data indicating that, over similar dose ranges, both agents are largely without effect in the rat social interaction and plus-maze tests (28). However, it should perhaps be noted that the latter study did report a very modest, though significant, increase in plus-maze anxiety with 0.6 mg/kg isoproterenol.

We believe that the present pattern of results can best be accommodated if it is assumed that different animal models are tapping qualitatively different facets of anxiety (18). It would, therefore, be predicted that these tests should vary in their sensitivity to drugs that elicit or inhibit different types of anxiety reaction. Of direct relevance in this context is the clinical distinction between generalized anxiety disorder and panic disorder [(1), but see also (24)], a distinction that is heavily based upon differences in pharmacotherapy and which raises the possibility that some drugs simply induce/exaccerbate feelings of anxiety while others precipitate full-blown panic attacks. The latter, in turn, may well explain some of the inconsistency of effect reported in humans following challenge with anxiety-provoking agents (4,41). It is, therefore, relevant to note that the elevated plus-maze test is remarkably sensitive to agents used to treat generalized anxiety disorder (i.e., benzodiazepines) but relatively insensitive to antipanic agents such as imipramine [for review: (47)]. Thus, the difference in profile currently obtained with FG 7142/PTZ (active) and sodium lactate/isoproterenol (inactive) would not be inconsistent with the proposal that the former increase a state akin to generalized anxiety, while the latter induce a panic-like effect to which the maze is insensitive. Further studies in this area are warranted.

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