Drug Development Research 7:61–73 (1986)

The Effects of Tofisopam, a 3,4-Benzodiazepine, in Animal Models of Anxiety, Sedation, and Convulsions

Sharon Pellow and Sandra E. File

MRC Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy, University of London, United Kingdom

ABSTRACT

Pellow, S., and S.E. File: The effects of tofisopam, a 3,4-benzodiazepine, in animal models of anxiety, sedation, and convulsions. Drug Dev. Res. **7**:61–73, 1986.

Tofisopam, a 3, 4-benzodiazepine classified as a nonsedative anxiolytic in man, had no effect in two animal tests of anxiety (the social interaction test and a punished drinking test; 10–50 mg/kg) and produced a dose-related reduction in exploratory and locomotor activity in the holeboard (5–100 mg/kg). These reductions were not antagonised by chlordiazepoxide (5 mg/kg), Ro 15-1788 (10–20 mg/kg), or picrotoxin (2 mg/kg); however, the reductions in head-dipping but not in motor activity or rears were antagonised by CGS 8216 (10 mg/kg). Tofisopam (10–50 mg/kg) had no anticonvulsant activity, but had proconvulsant activity, with picrotoxin and pentylenetetrazole. In contrast, tofisopam (50–100 mg/kg) antagonised the convulsions induced by the 1, 4-benzodiazepine Ro 5-4864.

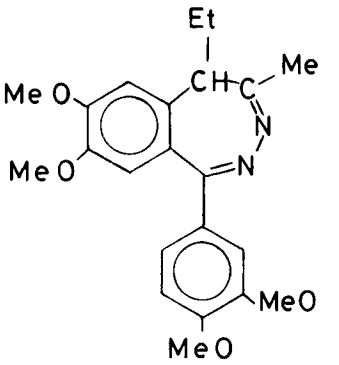
Key words: tofisopam, anxiety, convulsions, exploration, locomotor activity

INTRODUCTION

Tofisopam is a 3,4-benzodiazepine (see Fig. 1) that, unlike most 1,4-benzodiazepines, has no ability to displace [^{3}H]benzodiazepines from their receptor sites. Instead, it enhances the affinity of [^{3}H]flunitrazepam binding to these sites in vivo [Saano and Urtti, 1982]. In contrast, it does not enhance the affinity of [^{3}H]β-carboline, 3-carboxylate ethyl ester (β-CCE), which led Saano [1982a] to propose that tofisopam does not affect the cerebellar type of benzodiazepine site for which β-CCE has some selectivity. Tofisopam, like other benzodiazepines, enhances the binding of [^{3}H]muscimol to GABA receptors in vitro [Saano, 1982b].

Received final version February 21, 1985; accepted April 10, 1985.

Address reprint requests to Dr. S. Pellow, Département de Pharmacologie, Faculté de Médecine Pitié-Salpétrière, Université Paris VI, 91 Boulevard de l'Hôpital, 75634 Paris Cédex 13, France.



Tofisopam

Fig. 1. The chemical structure of tofisopam.

The mechanisms by which tofisopam is able to affect both benzodiazepine and GABA receptors in the CNS are unknown.

Tofisopam has long been classified as an effective compound for the treatment of clinical anxiety and one that is lacking in sedative side effects [Varady et al., 1975; Goldberg and Finnerty, 1979; Kanto et al., 1982]. However, preclinical data on this compound are lacking, and its profile in animal tests of anxiety and sedation has not been extensively investigated. It is possible that such investigations in rodents, and in particular the pattern of its interactions with other drugs, would lead to an indication of the sites that mediated the behavioural effects of this unusual compound.

The effects of tofisopam in the present study were investigated in two animal tests that have proven sensitive to the anxiolytic effects of benzodiazepines and novel anxiolytic compounds: the social interaction test [File and Hyde, 1979; File, 1980] and the punished drinking test [Vogel et al., 1971]. Its effects in both tests were compared to those of chlordiazepoxide, a typical benzodiazepine. In the social interaction test, its effects were investigated both acutely and chronically; with benzodiazepines, an anxiolytic effect is observed only after chronic treatment [File and Hyde, 1979].

The effects of tofisopam on exploratory head-dipping and spontaneous locomotor activity in the rat were examined in the holeboard test [File and Wardill, 1975], which is a very sensitive test for identifying potential sedative activity. Its effects were again compared to those of chlordiazepoxide. The effects of tofisopam in this test were examined in combination with several of the compounds in an attempt to identify the receptor sites that might mediate its effects: 1) chlordiazepoxide, a typical 1, 4-benzodiazepine that can antagonise the effects in the holeboard of PK 9084, a phenylquinoline believed to act at benzodiazepine receptors [File, 1983]. 2) Ro 15-1788, classified as a benzodiazepine receptor antagonist [Hunkeler et al., 1981] that can antagonise the effects of benzodiazepines in the holeboard [File et al., 1982, 1985; 3) CGS 8216, an "inverse agonist" at benzodiazepine receptors [Yokoyama et al., 1982] that also can antagonise the effects of benzodiazepines in the holeboard [File and Lister, 1983a; File et al., 1985]; and 4) picrotoxin, which binds to the picrotoxinin site on the GABA-benzodiazepine receptor complex in the CNS [see Olsen, 1982].

Finally, tofisopam was investigated for possible anticonvulsant or proconvulsant properties in combination with three convulsant compounds: picrotoxin and pentylenetetrazole, both believed to act at the picrotoxin site [see Olsen, 1982], and the convulsant 1,4-benzodiazepine Ro 5-4864, which has very little affinity for "classical" CNS benzodiazepine receptors but high affinity for peripheral-type benzodiazepine binding sites [Braestrup and Squires, 1977;

Schoemaker et al., 1982] and which also has an unidentified activity at the GABA-benzodiazepine receptor complex in the CNS [see Pellow and File, 1984a]. Doses of tofisopam were selected on the basis of pilot studies in this laboratory and on the basis of other studies [M. Briley, personal communication; Petocz and Kosoczky, 1975].

MATERIALS AND METHODS

Animals

Animals in the social interaction, punished drinking, and holeboard tests were male hooded Lister rats (Olac Ltd., Bicester, U.K.) weighing 150–200 g. Rats were singly housed for 5 days prior to the experiment in a room with an 11 hr light:13 hr dark cycle and allowed free access to food and water. For the social interaction test, rats were allocated to pairs on the basis of weight, i.e., no more than 5 g difference in the weights of partners was allowed.

Drugs

Tofisopam, diazepam, Ro 15-1788, Ro 5-4864, and CGS 8216 were suspended in distilled water with a drop of Tween 20. Chlordiazepoxide (CDP, Roche Products, U.K.), pentylenetetrazole (PTZ: Sigma, St. Louis, Missouri) and picrotoxin (Sigma) were dissolved in distilled water. All drugs were injected i.p. 30 min before testing in concentrations to give an injection volume of 2 ml/kg in rats and 4 ml/kg in mice.

The Social Interaction Test

Procedure. For details of apparatus, see File [1980]. On day 1 of testing, rats were tested in the low light, unfamiliar test condition. On day 2, animals were individually familiarised with the test arena (undrugged), and on day three they were again tested in the low light, familiar test condition. On the test days, pairs of rats were placed in the centre of the test arena, and the duration of their social interaction was scored for 7.5 min [File, 1980]. Rats were tested in an order randomised for drug treatment between 0730 and 1300 hr.

Pairs of rats were randomly allocated to the following treatment groups: experiment 1: vehicle control, tofisopam (10, 25, or 50 mg/kg), n = 9 per group; experiment 2: a) vehicle control, tofisopam (10, 25, or 50 mg/kg), b) vehicle control, chlordiazepoxide (5 mg/kg). In this experiment, all drugs were administered once daily for 5 days prior to testing. Both members of a pair always received the same drug treatment and were injected intraperitoneally 30 min before testing.

Statistics. Data from the social interaction test were analysed by analysis of covariance (ANCOVA) with drug treatment as independent factors. This allows the determination of the extent to which changes in social interaction scores are independent of changes in locomotor activity and vice versa. Two ANCOVAs were therefore performed, one with social interaction as the dependent variable and motor activity as the covariate and one with motor activity as the dependent variable and social interaction as the covariate. Adjusted means from the ANCOVA are given in brackets below the raw scores.

The Punished Drinking Test

Apparatus. The experimental chamber was a rectangular box 27 × 19.5 × 18 cm with transparent plastic sides, a wooden top, and an 18-bar grid floor. Through a hole in one wall, the rat had access to a stainless steel drinking spout (like that in the home cage) that protruded 1 cm into the cage and was connected to a plastic water bottle. The grid floor was connected via a scrambler to a shock generator (Lafayette). Scrambled electric shocks (0.17 mA, 0.5 sec duration) were delivered to the rat's feet by an observer.

Procedure. Testing took place over 2 days. On the first day, after being deprived of water (for the first time) for 23 hr, each animal was placed individually in the test chamber to

familiarise him with the environment and was left for 15 min. The rat was then returned to his home cage and allowed a further 2-hr access to water before being deprived for a further 23 hr. The second day was the test day. Each animal received an i.p. injection and was returned to his home cage from which the food had been removed. Thirty minutes later, he was placed in the experimental chamber for a 5-min test session. The animals were allowed 15 sec free drinking before they received their first shock. After each subsequent 3 sec drinking, a shock was delivered to the feet. The observer scored the latency to the first lick, and the total number of shocks received by each rat in the 5 min test period. Independent groups were run as unpunished controls; these rats received exactly the same treatment as the punished rats except that no shocks were delivered during the test session, and the observer recorded the amount of time spent drinking instead of the number of shocks.

Animals were randomly allocated to the following treatment groups: 1) vehicle control, tofisopam (10 or 25 mg/kg); and 2) vehicle control, CDP (5 or 7.5 mg/kg), n = 6.

Statistics. Data were analysed by one-way analysis of variance (ANOVA) with drug treatment as the factor. Posthoc comparisons with control groups were made using Dunnett's t tests.

The Holeboard Test

Procedure. For apparatus details, see File and Wardill [1975] and File [1983]. Rats were tested in an order randomised for drug treatment between 0700 and 1300 hr. They were placed singly in the centre of the holeboard and given a 7.5-min trial, during which the following measures were recorded via computer: the number of head-dips, the time spent head-dipping, a locomotor activity score, and the number of rears. Rats were randomly allocated to the following treatment groups: experiment 1: a) vehicle control, tofisopam (5, 10, 25, 50, and 100 mg/kg), n = 9; b) control, CDP (5 and 10 mg/kg), n = 8; experiment 2: vehicle control, tofisopam (25 mg/kg) alone and in combination with chlordiazepoxide (CDP; 5 mg/kg) or Ro 15-1788 (10 or 20 mg/kg); experiment 3: vehicle control, tofisopam (25 mg/kg) alone and in combination with CGS 8216 (10 mg/kg) or picrotoxin (2 mg/kg). All rats received two i.p. injections before testing.

Statistics. Data were analysed by one-way ANOVA with drug treatment as the factor. Posthoc comparisons between drug groups and control were made using Dunnett's t tests and between drug groups using Duncan's multiple range tests.

Convulsions

Animals. Animals were male Tuck No. 1 mice (Tuck and Sons, Battlesbridge, U.K.) weighing 25–30 g. Mice were housed in groups of 30 in each room with an 11 hr light:13 hr dark cycle and allowed free access to food and water.

Procedure. Pilot experiments established convulsant doses of picrotoxin, PTZ, and Ro 5-4864 and subconvulsant doses of picrotoxin and PTZ. Convulsant doses were defined as the lowest dose to produce myoclonic jerks and convulsions in all mice. Subconvulsant doses were defined as the highest doses that caused no myoclonic jerks or convulsions in the mice. Mice were tested between 1400 and 1800 hr. After injection of the convulsant compounds each mouse was placed in an individual box and observed for 30 min. The latency to the first myoclonic jerk (sudden extension of the forelimbs) and the latency to the first full tonic-clonic convulsion (extension and contraction of fore- and hindlimbs) were recorded. Mice were randomly allocated to the following treatment groups (n = 8): experiment 1: picrotoxin (3 or 6 mg/kg) alone and in combination with tofisopam (10, 25, or 50 mg/kg), or picrotoxin (6 mg/kg) in combination with diazepam (2 mg/kg); experiment 2: PTZ (30 or 60 mg/kg) alone and in combination with tofisopam (10, 25, or 50 mg/kg) or PTZ (60 mg/kg) in combination with diazepam (2 mg/kg); Experiment 3: Ro 5-4864 (50 mg/kg) alone and in combination with tofisopam (50 or 100 mg/kg) or diazepam (2mg/kg). Tofisopam was injected 30 min before convulsant compounds.

Statistics. Data were analysed by t test (latency data) and by Fisher's exact probability test (frequency data).

RESULTS

The Social Interaction Test

Experiment 1. Analysis of covariance with social interaction as the dependent variable and motor activity as the covariate showed that the social interaction of animals increased significantly with increasing familiarity [$F(1,28) = 64.6$, $P < .0001$]. Tofisopam had no significant overall effect on social interaction ($F(3,28) = 1.45$) and did not have selective effects on either test condition [$F(3,28) = 0.66$] (see Table 1). However, ANCOVA with motor activity as the dependent variable and social interaction as the covariate showed that tofisopam significantly reduced locomotor activity [$F(3,28) = 2.94$, $P = .05$] and that this effect was not dependent on the test condition [$F(3,28) = 1.47$].

Experiment 2. 1. ANCOVA with social interaction as the dependent variable and motor activity as the covariate showed that tofisopam had no significant overall effect on social interaction [$F(3,25) = 0.57$] (see Table 2). There was a significant familiarity effect [$F(1,25) = 28.37$, $P < .0001$] showing that social interaction increased significantly from LU to LF conditions. However, a nonsignificant drug × familiarity interaction [$F(3,25) = 0.47$) indicated that tofisopam had no selective effects in either condition. Analysis of covariance with motor activity as the dependent variable and social interaction as the covariate showed that tofisopam had no significant overall effect on motor activity [$F(3,25) = 2.6$ $P < .08$], (see Table 2).

TABLE 1. The Effects of Acute Tofisopam on Social Interaction*

		Tofisopam (mg/kg)		
Conditions	Control	10	25	50
Social				
LU[a]	147 ± 18.1	159 ± 23.4	82 ± 12.2	63 ± 15.9
LF[b]	375 ± 26.5	284 ± 33.7	209 ± 54.4	131 ± 33.8
Motor				
LU	775 ± 12.2	743 ± 25.3	629 ± 82.7	541 ± 96.1
LF	790 ± 21.5	669 ± 39.5	539 ± 40.6	259 ± 55.5

*Mean ± SEM time(s) spent in active social interaction and locomotor activity score for pairs of rats given a 7.5-min trial, 30 min after injection of control or tofisopam.
[a]Low light, unfamiliar condition.
[b]Low light, familiar condition.

TABLE 2. Effects of Chronic Tofisopam on Social Interaction*

		Tofisopam (mg/kg)				Chlordiaz-epoxide
Conditions	Control	10	25	50	Control	(5 mg/kg)
Social						
LU[a]	150 ± 24.2	170 ± 18.8	144 ± 26.1	135 ± 26.1	148 ± 13.64	262 ± 28.72
LF[b]	267 ± 28.6	273 ± 27.6	210 ± 43.0	180 ± 36.1	369 ± 37.10	311 ± 45.72
Motor						
LU	501 ± 38.7	470 ± 27.0	361 ± 40.0	401 ± 48.3	522 ± 23.61	506 ± 9.53
LF	511 ± 30.3	500 ± 34.7	411 ± 5.4	332 ± 58.1	551 ± 10.05	581 ± 31.56

*Mean ± SEM time(s) spent in active social interaction and locomotor activity score for pairs of rats given a 7.5-min trial 30 min after injection of control or tofisopam or chlordiazepoxide for 5 days.
[a]Low light, unfamiliar condition.
[b]Low light, familiar condition.

There was no significant drug × familiarity interaction [$F(3,25) = 2.54$, $P < .08$] showing that tofisopam did not have selective effects in either of the two test conditions.

2. ANCOVA showed that there was a significant familiarity × drug effect for CDP (5 mg/kg) [$F(1,9) = 5.04$, $P = .05$], and posthoc analysis showed that CDP elevated social interaction selectively in the unfamiliar test condition ($P < .05$) but not in the familiar condition (see Table 2). There was no significant familiarity × drug effect on motor activity [$F(1,9) = 0.04$].

The Punished Drinking Test

Punished drinking. 1.ANOVA showed no significant drug effect on latency [$F(2,17) = 0.89$]. There was a significant overall effect on the number of shocks taken [$F(2,17) = 25.88$, $P < .0001$]; posthoc analysis showed that groups treated with both 10 and 25 mg/kg tofisopam differed significantly from controls ($P < .01$; see Table 3).

Unpunished drinking. There was a significant overall drug effect on the latency to first lick [$F(2,15) = 7.79$, $P < .005$], and post hoc analysis showed that at 25 mg/kg tofisopam caused a significant increase in the latency to the first lick ($P < .01$, see Table 3). There was also a significant drug effect on time spent drinking [$F(2,15) = 20.5$, $P < .0001$], and posthoc analysis showed that both at 10 and 25 mg/kg tofisopam increased unpunished drinking ($P < .05$, see Table 3).

Unpunished drinking. 2. ANOVA showed that CDP (5–7.5 mg/kg) had no significant effect on unpunished drinking [$F(2,12) = 0.23$], but there was a significant increase in the latency to the first lick [$F(2,12) = 8.57$, $P < .005$], probably indicative of sedative effects.

Punished drinking. There was a significant increase in the number of shocks received by the rats treated with CDP (5–7.5 mg/kg) compared to controls [$F(2,12) = 5.88$, $P < .05$] but no significant increase in the latency to lick [$F(2,12) = 0.9$].

The Holeboard Test

Experiment 1. 1.ANOVA showed that tofisopam significantly reduced the number of head-dips [$F(5,54) = 23.0$, $P < .0001$], the time spent head-dipping [$F(5,54) = 13.5$, $P < .0001$], the locomotor activity [$F(5,54) = 32.7$ $P < .0001$], and the number of rears [$F(5,54) = 29.7$, $P < .0001$). The doses to do so significantly are shown in Table 4.

2.ANOVA showed that CDP (5–10 mg/kg) significantly reduced the number of head-dips [$F(2,21) = 9.38$, $P < .005$], the locomotor activity score [$F(2,12) = 5.36$, $P < .05$], and the number of rears [$F(2,12) = 11.55$, $P < .0005$] but not the time spent head dipping [$F(2,12) = 2.44$]. Doses to do so significantly are shown in Table 4.

TABLE 3. Effects of Tofisopam in the Vogel Punished Drinking Test*

		Unpunished controls		Punished groups	
Group	Mg/kg	Latency	Time	Latency	Number
Control		11.5 ± 2.8	131.1 ± 6.8	1.3 ± 0.9	23.0 ± 3.5
Tofisopam	10	10.0 ± 3.2	179.3 ± 4.5[a]	4.1 ± 1.5	57.1 ± 2.2[a]
Tofisopam	25	27.0 ± 3.9[b]	173.5 ± 5.7[a]	4.0 ± 2.0	53.8 ± 4.5[b]
Control		12.0 ± 2.3	127.2 ± 14.5	3.8 ± 2.1	19.4 ± 2.8
Chlordiaze-poxide	5	12.8 ± 2.9	121.8 ± 11.9	2.2 ± 1.2	50.8 ± 5.1
Chlordiaze-poxide	7.5	28.0 ± 3.8	136.2 ± 17.9	9.4 ± 6.4	44.2 ± 10.2

*Mean ± SEM latency time(s) spent drinking and number of shocks taken by rats given a 5-min drinking test in either unpunished or punished conditions (see text) 30 min after injection with tofisopam or chlordiazepoxide.

[a]$P < .05$.

[b]$P < .01$.

TABLE 4. Effects of Tofisopam in the Holeboard*

Group	Mg/kg	Number	Time	Motor	Rears
Control		23.3 ± 1.79	25.7 ± 1.59	224.3 ± 17.38	52.8 ± 3.78
Tofisopam	5	17.6 ± 1.85	16.4 ± 2.73	225.8 ± 18.57	56.8 ± 6.19
	10	14.3 ± 1.52	13.2 ± 2.40	238.0 ± 10.20	53.7 ± 2.28
	25	7.1 ± 1.50	11.5 ± 2.40	117.9 ± 18.88	28.2 ± 4.92
	50	3.9 ± 1.17	4.7 ± 1.16	68.5 ± 7.23	7.6 ± 1.80
	100	3.6 ± 2.08	4.6 ± 2.24	41.5 ± 15.86	9.2 ± 4.45
Control		20.4 ± 2.69	21.4 ± 3.24	225.5 ± 40.12	46.6 ± 4.71
Chlordiaze-	5	19.2 ± 1.97	21.2 ± 2.01	209.7 ± 40.71	35.4 ± 6.33
poxide	10	7.4 ± 2.32	12.6 ± 4.11	80.3 ± 17.06	14.6 ± 2.45

*Mean ± SEM number of head-dips, time(s) spent head-dipping, locomotor activity, and number of rears for rats given a 7.5-min test in the holeboard 60 min after injection with vehicle, tofisopam, or chlordiazepoxide.

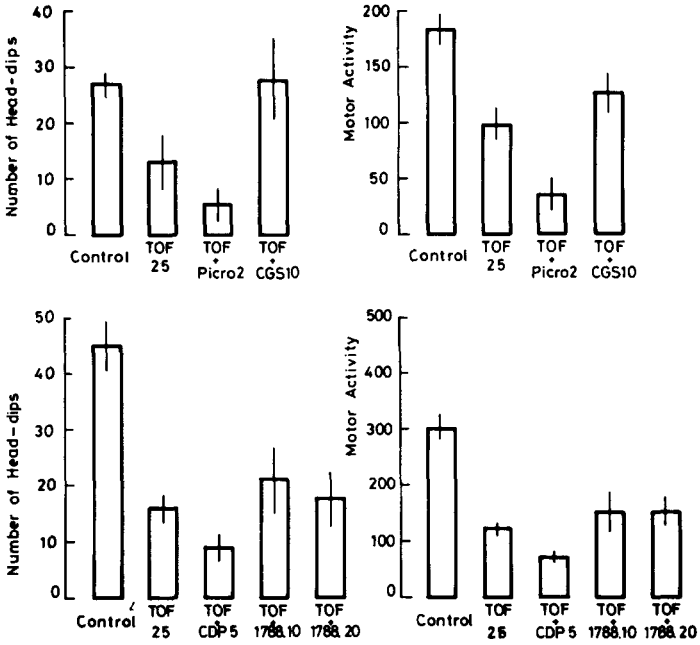


Fig. 2. Mean (± SEM) number of head-dips and locomotor activity score for rats given a 7.5-min test in the holeboard, 30 min after injection with tofisopam (25 mg/kg), alone or in combination with picrotoxin (2 mg/kg), CGS 8216 (10 mg/kg), chlordiazepoxide (5 mg/kg), or Ro 15-1788 (10 or 20 mg/kg).

Experiment 2. 1. ANOVA showed a significant overall drug effect on the number of head-dips [$F(4,38) = 12.02$, $P < .0001$]; Dunnett's tests showed that tofisopam (25 mg/kg) significantly reduced the number of head-dips ($P < .01$) both alone and in combination with CDP (5 mg/kg) or Ro 15-1788 (10 or 20 mg/kg; see Fig. 2). No combination group differed from tofisopam when given alone. There was a significant drug effect on time spent head-dipping [$F(4,38) = 5.3$ $P < .005$], and post hoc analysis showed that all groups given tofisopam spent significantly less time head-dipping than controls ($P < .05$; see Table 5). None of the combination groups differed significantly from the group treated with tofisopam alone. There was a significant drug effect on locomotor activity [$F(4,38) = 19.9$ $P < .0001$];

TABLE 5. Antagonism of the Effects of Tofisopam in the Holeboard*

Group	Mg/kg	Time	Rears
Control		46.9 ± 4.41	36.5 ± 3.50
Tofisopam	25	23.7 ± 3.37	13.7 ± 2.46
+Chlordiazepoxide	5	16.1 ± 3.02	1.1 ± 0.34
+Ro15-1788	10	25.7 ± 3.06	10.7 ± 4.37
+Ro15-1788	20	29.1 ± 6.31	14.4 ± 3.72
Control		17.6 ± 2.84	14.5 ± 3.92
Tofisopam	25	9.8 ± 2.88	5.7 ± 2.25
+CGS 8216	10	14.0 ± 1.94	8.5 ± 2.09
+Picrotoxin	2	4.1 ± 2.70	2.9 ± 1.60

*Mean ± SEM time spent head-dipping and number of rears for rats given a 7.5-min test in the holeboard 30 min after i.p. injection of tofisopam alone and in combination with chlordiazepoxide, Ro 15-1788, CGS 8216, or picrotoxin.

again, all treatment groups had reduced locomotor activity scores compared to controls ($P < .01$; see Fig. 2), but no groups differed from the group given tofisopam alone. There was an overall drug effect on the number of rears [$F(4,38) = 21.15$, $P < .0001$]; all treatment groups made a significantly reduced number of rears compared to controls ($P < .01$, see Table 5: The group given CDP (5 mg/kg) in combination with tofisopam also had significantly lower scores than animals treated with tofisopam alone ($P < .05$; Table 5).

2.ANOVA showed a significant overall drug effect on the number of head-dips [$F(3,28) = 4.07$, $P < .05$]; posthoc analysis showed that tofisopam alone (25 mg/kg) significantly decreased head-dipping ($P < .05$: This was significantly reversed by CGS 8216 (10 mg/kg, $P < .05$; see Fig. 2). The group given the combination of tofisopam and picrotoxin was significantly different from controls ($P < .01$) but not from the tofisopam alone group. There was a significant overall drug effect on the time spent head-dipping [$F(3,28 = 6.05$, $P < .005$]; posthoc analysis showed that tofisopam alone significantly reduced the time spent head-dipping ($P < .01$): This was significantly antagonised by CGS 8216 (10 mg/kg, $P < .05$; see Table 5). The group given the combination of tofisopam and picrotoxin was significantly different from controls ($P < .01$) but not from the tofisopam alone group. There was a significant overall drug effect on locomotor activity [$F(3,28) = 13.57$, $P < .001$]; tofisopam alone significantly reduced activity, an effect that was significantly enhanced by picrotoxin ($P < .01$, see Fig. 2) but not affected by CGS 8216. There was a significant overall drug effect on the number of rears [$F(3,28) = 3.59$, $P < .05$]; tofisopam alone significantly reduced rearing ($P < .01$). This was unaffected either by picrotoxin or CGS 8216 (see Table 5).

Convulsions

Experiment 1. Picrotoxin (3mg/kg) caused no myoclonic jerks or convulsions in any mice; however, in combination with tofisopam (25–50 mg/kg), a significant number of mice showed myoclonic jerks ($P < .05$, $P < .01$, respectively), and at 25 mg/kg tofisopam a significant number of mice showed convulsions ($P < .05$; see Table 6). With picrotoxin (6 mg/kg), all mice showed myoclonic jerks and convulsions; and diazepam (2 mg/kg) suppressed myoclonus and convulsions in all mice; however, tofisopam (10–50 mg/kg) did not affect the number and severity of the convulsions that were observed. An examination of the latency data (Table 6) shows that tofisopam significantly reduced the latency to myoclonus (25 mg/kg, $P < .05$; 50 mg/kg, $P < .001$) and to convulse (10 mg/kg, $P < .05$; 25 and 50 mg/kg, $P < .001$), consistent with the proconvulsant properties of tofisopam observed with subconvulsant doses of picrotoxin.

Experiment 2. PTZ (30 mg/kg) caused no myoclonic jerks or convulsions in any mice; however, in combination with tofisopam (25–50 mg/kg) a significant number of mice showed

TABLE 6. Effects of Tofisopam on Picrotoxin- and Pentylenetetrazole-Induced Seizures*

Drug	Mg/kg	Latency to myoclonus	Latency to convulse	No. myoclonus	No. convulsing
A. Picrotoxin	3	—	—	1/8	0/8
+ Tof[a]	10	—	—	0/8	0/8
+ Tof	25	952 ± 62.0	1318 ± 88.3	6/8[g]	4/8[g]
+ Tof	50	987 ± 94.2	1207 ± 53.4	8/8[h]	3/8
PTZ[b]	30	—	—	0/8	0/8
+ Tof	10	—	—	0/8	0/8
+ Tof	25	731 ± 48.9	1162 ± 224.2	7/8[h]	3/8
+ Tof	50	868 ± 66.3	1366 ± 138.0	8/8[h]	2/8
B. Picrotoxin	6	526 ± 17.1	662 ± 14.9	8/8	8/8
+ Tof	10	512 ± 41.6	604 ± 25.7[d]	8/8	8/8[c]
+ Tof	25	429 ± 33.9[d]	491 ± 31.8[f]	8/8	8/8[c]
+ Tof	50	344 ± 27.3[f]	416 ± 27.6[f]	8/8	8/8[c]
+ DZ	2	—	—	0/6[h]	0/6[h]
PTZ	60	90 ± 4.0	203 ± 8.3	8/8	8/8
+ Tof	10	90 ± 6.3	179 ± 7.9	8/8	8/8[c]
+ Tof	25	76 ± 7.0	159 ± 9.1[e]	8/8	8/8[c]
+ Tof	50	83 ± 6.9	157 ± 6.5[f]	8/8	8/8[c]
+ DZ	2	—	—	0/6[h]	0/6[h]

*Latency to first myoclonus or convulsion and number of mice showing myoclonus or convulsions after (A) subconvulsant and (B) convulsant doses of picrotoxin and pentylenetetrazole alone and in combination with tofisopam or diazepam. All drugs were injected i.p.; tofisopam and diazepam were given 30 min before the convulsants.

[a]Tofisopam.

[b]Pentylenetetrazole.

[c]These animals had several convulsions within the 30-min observation period; only one is usually observed with these doses of picrotoxin and PTZ.

[d]$P < .05$ Student's t test (latency).

[e]$P < .01$ Student's t test (latency).

[f]$P < .001$ Student's t test (latency).

[g]$P < .05$ Fisher-Yates exact probability test (number).

[h]$P < .01$ Fisher-Yates exact probability test (number).

myoclonic jerks ($P < .01$), but there was not a significant increase in the number of mice to have convulsions (Table 6). With PTZ (60 mg/kg), all mice showed myoclonic jerks and convulsions; diazepam (2 mg/kg) prevented myoclonus and convulsions in all mice, but tofisopam (10–25 mg/kg) did not significantly affect the number of mice showing these behaviours (Table 6). However, as with picrotoxin, tofisopam increased the number and severity of the convulsions observed in these mice. The latency data show that tofisopam significantly reduced the latency to convulse (25 mg/kg, $P < .01$; 50 mg/kg, $P < .001$; Table 6), consistent with the proconvulsant properties of tofisopam at these doses with subconvulsant doses of PTZ.

Experiment 3. Ro 5-4864 (50 mg/kg) produced myoclonus and convulsions in all mice; diazepam (2 mg/kg) suppressed signs of both behaviours in all mice. Tofisopam (100 mg/kg) significantly reduced the number of mice to convulse ($P < .05$), but all mice had myoclonic jerks (Table 7). At 50 and 100 mg/kg, tofisopam significantly increased the latency to convulse ($P < .01$; Table 7).

DISCUSSION

Tofisopam (10–50 mg/kg) acutely or after 5 days of pretreatment has no effect in the social interaction test, which has proven sensitive to the anxiolytic effects of several benzodi-

TABLE 7. Effects of Tofisopam on Ro5-4864-Induced Convulsions*

Drug	Mg/kg	Latency to myoclonus(s)	Latency to convulse(s)	No. myoclonus	No. convulsing
Ro5-4864	50	478 ± 59.9	514 ± 66.9	8/8	8/8
+ Tofisopam	50	677 ± 80.7	1293 ± 142.0[a]	8/8	6/8
+ Tofisopam	100	794 ± 172.7	1349 ± 210.6[a]	8/8	4/8[b]
+ Diazepam	2	—	—	0/8	0/8[c]

*Latency to first myoclonus or convulsion and number of mice showing myoclonus or convulsions after a convulsant dose of Ro5-4864 alone and in combination with Tofisopam or diazepam. Tofisopam and diazepam were given i.p. 30 min before i.p. injection of Ro5-4864.
[a]$P < .01$ Student's t test.
[b]$P < .05$ Fisher-Yates exact probability test.
[c]$P < .01$ Fisher-Yates exact probability test.

azepines and nonbenzodiazepine putative anxiolytics [File and Hyde, 1979; File, 1982; File and Pellow, 1985a] and has been well validated for both anxiolytc and anxiogenic drug actions [see File, 1980; Pellow and File, 1984c]. In contrast, chlordiazepoxide (5 mg/kg) given after 5 days of pretreatment caused a significant increase in the time spent in active social interaction. Whereas tofisopam produced an increase in the number of shocks a rat would receive during a 5-min punished drinking trial, there was also an increase in unpunished drinking. This is in contrast to the results with chlordiazepoxide, which produced a selective increase in punished drinking only (5–7.5 mg/kg). In that tofisopam affects water intake in control conditions, its effect during the punished period cannot be described as anxiolytic but might be due to direct effects of tofisopam on drinking behaviour. The results from these two tests are in agreement with those of Stenger et al. [in preparation], who found that tofisopam had no activity alone in the Geller-Seifter test. However, tofisopam was able to enhance the anxiolytic efficacy of diazepam in both the punished drinking test [Stenger et al., 1983] and the Geller-Seifter conflict test [Stenger, personal communication].

It would not be instructive to investigate higher doses of tofisopam; significant reductions in spontaneous behaviour after acute treatment are already present at 25 mg/kg, and even when some tolerance has developed to these sedative effects after 5 days of pretreatment, there is no hint of an anxiolytic action in the social interaction test that might have been masked by sedation. This clearly contrasts with several reports suggesting that tofisopam is an effective anxiolytic compound in man [Varady et al., 1975; Goldberg and Finnerty, 1979; Kanto et al., 1982].

That tofisopam has sedative properties in the rat (defined by reduced spontaneous locomotor activity in the holeboard) is also in contrast to the clinical results suggesting that this compound lacks sedative side effects. However, these results are consistent with those of Petocz and Kosoczky [1975], who obtained sedative effects in cats (direct observation, 12 mg/kg); reduced rearing, grooming, and locomotion in rats (100 mg/kg p.o.); and potentiation of pentobarbitone sleeping time in mice (25–100 mg/kg, p.o.). In these effects, tofisopam resembles acutely administered chlordiazepoxide (5–10 mg/kg), which also significantly decreased all behaviour in the holeboard.

The results from the above tests of anxiety and sedation show that tofisopam has a very different profile in rodents than that reported in man. Possibly this is the result of differing metabolism in these two species; however, no detailed information is available on this subject.

Which sites of action are mediating the sedative effects of tofisopam? Chlordiazepoxide (5 mg/kg), which is able to antagonise the reduced exploration produced by PK 9084 [File, 1983], enhanced the reductions in all measures produced by tofisopam. Ro 15-1788, which increases head-dipping when given alone and antagonises only the effects of drugs believed to exert their effects at classical CNS benzodiazepine receptors [File et al., 1982, 1985; File and Pellow, 1984a, 1985a], failed (10–20 mg/kg) to antagonise the sedative effects of tofisopam.

This makes it unlikely that tofisopam is exerting its sedative effects through a direct action at the benzodiazepine receptor, which is consistent with biochemical evidence suggesting that it has no direct effect there [Saano, 1982a].

Interestingly, CGS 8216 (10 mg/kg) reversed the reductions in head-dipping, but not in motor activity or rearing, produced by tofisopam. This compound, which decreases head-dipping when given alone, also antagonises the reductions in behaviour produced by chlordiazepoxide in the holeboard [File and Lister, 1983a]. However, it is by no means certain that CGS 8216 is able to antagonise these effects through an action at the benzodiazepine receptor; a variety of evidence now suggests that the intrinsic actions seen with doses of this compound, approximately ten times higher than those necessary to antagonise the effects of benzodiazepines [Yokoyama et al, 1982], can be mediated by nonbenzodiazepine binding sites [File and Lister, 1983b; File and Pellow, 1984b, 1985b; Pellow et al., 1984]. The most likely possibility in the light of the combinations of tofisopam with Ro 15-1788 is that tofisopam and CGS 8216 interact at some other, as yet unidentified, site in the CNS. Further biochemical studies are clearly necessary to investigate this possibility.

The enhanced reductions in spontaneous behaviour observed with the combination of picrotoxin and tofisopam are likely to be the result of convulsant activity; myoclonic jerks were observed in several of the rats given both drugs, and indeed subsequent experiments showed that tofisopam has proconvulsant activity with this compound. Tofisopam (10–50 mg/kg) had no anticonvulsant activity against picrotoxin and pentylenetetrazole in contrast to diazepam, which was effective against both agents at 2 mg/kg. Briley et al. [1984], however, found that tofisopam at these doses enhanced the anticonvulsant efficacy of diazepam against these and other convulsants. In contrast, tofisopam (10–50 mg/kg) had weak proconvulsant activity when combined with subthreshold doses of these convulsants. This is in keeping with observations made by Petocz and Kosoczky [1975] that tofisopam decreased the threshold for pentylenetetrazole convulsions to some extent. This proconvulsant effect could reflect an interaction of tofisopam with the picrotoxinin site in the CNS; Ramanjaneyulu and Ticku [1984] have recently reported that tofisopam (IC_{50} = 160 μM) was able to inhibit the binding of [^{35}S]t-butylbicyclophosphorothionate (TBPT) to these sites.

In contrast to its interactions with picrotoxin and pentylenetetrazole, tofisopam (50 mg/kg) was able to block the convulsions caused by the 1,4-benzodiazepine Ro 5-4864. In this effect it resembles diazepam (2 mg/kg), although it is clearly less potent than the latter compound. Ro 5-4864 has very high affinity for the peripheral type of benzodiazepine site in the periphery and the brain [Braestrup and Squires, 1977; Schoemaker et al., 1982]. Interestingly, tofisopam is also able to displace the binding of [^{3}H]Ro 5-4864 from peripheral-type binding sites in the CNS [M. Briley, personal communication]. However, it is possible that the convulsant actions of Ro 5-4864 are mediated by a site on the GABA-benzodiazepine receptor complex [see Pellow and File, 1984a,b], and so the possibility remains that tofisopam can also exert its anticonvulsant activity at this complex.

In conclusion, the behavioural profile of tofisopam in rodents identifies it as a sedative compound with mixed anti- and proconvulsant properties that has no anxiolytic activity. As with several other compounds that have mixed anti-and proconvulsant properties [see Pellow, 1985, for review], this pattern of effects makes tofisopam a particularly interesting compound to study the neural mechanisms underlying seizure activity. Although both behavioural and biochemical lines of evidence agree that the effects of tofisopam are unlikely to be mediated by classical CNS benzodiazepine receptors, more detailed biochemical investigation would be necessary before clear alternate sites of action can be specified. Recent studies have shown that tofisopam might possess activity at dopamine receptors in the CNS [Chopin et al., 1982], which might contribute to its unusual pharmacological profile.

ACKNOWLEDGMENTS

We are grateful to Pierre Fabre Médicament for the gift of tofisopam and for providing financial support for these studies. Thanks are also due to CIBA-GEIGY and Hoffman-La

Roche for providing CGS 8216, and Ro 5-4864, and Ro 15-1788, to Peter Mabbutt for expert technical assistance, and to Philippe Chopin for helpful reading of the manuscript and for discussions on tofisopam. S.E.F. is a Wellcome Trust Senior Lecturer.

REFERENCES

Braestrup, C., and Squires, R.F.: Specific benzodiazepine receptors in rat brain characterised by high-affinity [^{3}H]-diazepam binding. Proc. Natl. Acad. Sci. USA **74**::3805–3809, 1977.

Briley, M., Charveron, M., Couret, E., and Stenger, A.: Tofisopam enhances the anticonvulsant activity of diazepam against some, but not all, convulsive agents. Br. J. Pharmacol. **82**[Suppl]:300P, 1984.

Chopin, P., Stenger, A., and Charveron, M.: Propriétés agonistes dopaminergiques d'une benzodiazépine 3,-4, le tofisopam. In: "Pharmacologie et Toxicologie Vétérinaires, Les Collogues de L'INRA 8." Paris; INRA ,1984, pp. 105–109.

File, S.E.: The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J. Neurosci. Meth. **2**:219–238, 1980.

File, S.E.: Animal anxiety and the effects of benzodiazepines. In Usdin, E., Skolnick, P., Tallman, J. F., Greenblatt, D., and Paul, S.M.: (eds): "Pharmacology of Benzodiazepines," London: Macmillan Press, 1982, pp. 355–364.

File, S.E.: Sedative effects of PK 9084 and PK 8165, alone and in combination with chlordiazepoxide. Br. J. Pharmacol **79**:219–223, 1983.

File, S.E., and Hyde, J.R.G.: A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilisers and of stimulants. Pharmacol. Biochem. Behav. **11**:65–69, 1979.

File, S.E., and Lister, R.G.: Ro 15-1788 and chlordiazepoxide each reverse the reduction in exploration caused by CGS 8216. Br. J. Pharmacol. **78**:95P, 1983a.

File, S.E., and Lister, R.G.: Interactions of ethyl-β-carboline-3-carboxylate and Ro 15-1788 with CGS 8216 in an animal model of anxiety. Neurosci. Lett. **39**:91–94, 1983b.

File, S.E., Lister, R.G., and Nutt, D.J.: Intrinsic actions of benzodiazepine antagonists. Neurosci. Lett. **32**:165–168, 1982.

File, S.E., and Pellow, S.: Behavioural effects of PK 8165 that are not mediated by benzodiazepine receptors. Neurosci. Lett. **50**:197–201, 1984a.

File S.E., and Pellow, S.: The anxiogenic action of FG 7142 in the social interaction test reversed by chlordiazepoxide and Ro 15-1788 but not by CGS 8216. Arch. Int. Pharmacodym. Ther. **271**:198–204, 1984b.

File, S. E. and Pellow, S.: The anxiolytic but not the sedative properties of tracazolate are reversed by the benzodiazepine receptor antagonist, Ro 15-1788. Neuropsychobiology (in press), 1985a.

File, S.E., and Pellow, S.: Chlordiazepoxide enhances the anxiogenic action of CGS 8216: Evidence for benzodiazepine withdrawal? Pharmac. Biochem Behav. **23**:33–36, 1985b.

File, S.E., Pellow, S., and Wilks, L.: The sedative effects of CL 218,872, like those of chlordiazepoxide, are reversed by benzodiazepine antagonists. Psychopharmacology **85**:295–300, 1985.

File, S.E., and Wardill, A.G: Validity of head-dipping as a measure of exploration in a modified holeboard. Psychopharmacologia **44**:53–59, 1975.

Goldberg, H.L., and Finnerty, R.J.: Comparative efficacy of tofisopam and placebo. Am. J. Psychiatr. **136**:196–199, 1979.

Hunkeler, W., Mohler, H., Pieri, P., Polc, P., Bonetti, E.P., Cumin, R., Schaffner, R. and Haefely, W.: Selective antagonists of benzodiazepines. Nature **290**:514–516, 1981.

Kanto, J., Kangas, L., Leppanen, T., Mansikka, M., and Sibakov, M.-L.: Tofisopam: A benzodiazepine derivative without sedative effect Int. J. Clin. Pharmacol. Ther. Toxicol. **20**:309–312, 1982.

Olsen, R.W.: Drug interactions at the GABA-receptor-ionophore complex. Annu. Rev. Pharmacol. Toxicol. **22**:245–277, 1982.

Pellow, S.: Can drug effects on anxiety and convulsions be separated? Neurosci. Biobehav. Rev. **9**:55–73, 1985.

Pellow, S., and File, S.E: Characteristics of an atypical benzodiazepine, Ro 5-4864. Neurosci. Biobehav. Rev. **8**:405–413, 1984a.

Pellow, S., and File, S.E: Behavioural actions of Ro 5-4864, a peripheral-type benzodiazepine? Life Sci. **35**:229–240, 1984b.

Pellow, S., and File, S.E.: Multiple sites of action for anxiogenic drugs: Behavioural, electrophysiological and biochemical correlations. Psychopharmacology **83**:304–315, 1984c.

Pellow, S., File, S.E., and Herberg, L.J.: Intracranial self-stimulation distinguishes between two benzodiazepine antagonists, Neurosci. Lett. **47**:173–177, 1984.

Petocz, L., and Kosoczky, I.: The main pharmacologocal characteristics of Grandaxin (tofisopam, Egyt-341). Ther. Hung. **23**:134–138, 1975.

Ramanjaneylulu, R., and Ticku, M.K.: Binding characteristics and interactions of depressant drugs with [^{35}S]t-butylbicyclophosphorothionate, a ligand that binds to the picrotoxinin site. J. Neurochem. **42**:221–229, 1984.

Saano, V.: Tofisopam selectively increases the affinity of benzodiazepine binding sites for [^{3}H]-flunitrazepam but not for [^{3}H]-β-carboline-3-carboxylic acid ethyl ester. Pharmacol. Res. Commun. **14**:971–981, 1982a.

Saano, V.: Tofisopam affects binding of [^{3}H]-muscimol to Gamma-amino-butyric acid receptors in rat and mouse brains. Pharmacol Res. Commun. **14**:745–757., 1982b.

Saano, V., and Urtti, A.: Tofisopam modulates the affinity of benzodiazepine receptors in the rat brain. Pharmac. Biochem. Behav. **17**:367–369, 1982.

Schoemaker, H., Bliss, M., and Yamamura, H.I.: Specific high-affinity saturable binding of [^{3}H]-Ro 5-4864 to benzodiazepine binding sites in the rat cerebral cortex. Eur. J. Pharmacol. **71**:173–175, 1982.

Stenger, A., Charveron, M., and Briley, M.: Tofisopam, a 3, 4-benzodiazepine, enhances the anticonflict activity of diazepam. Naunyn-Schmiedeberg Arch. Pharmacol. **R 24**:94, 1983.

Varady, G., Bolla, K., and Sebo, J.: The clinical evaluation of Grandaxin used in the treatment of outpatients (a multicentric study). Ther. Hung. **23**:153–158, 1975.

Vogel, J.R., Beer, B., and Clody, D.E.: A simple and reliable conflict procedure for testing antianxiety agents. Psychopharmacology **21**:1–7, 1971.

Yokoyama, N., Ritter, B., and Neubert, A.D.: 2-Arylpyrazolo 4, 3-cquinolin-3-ones: novel agonist, partial agonist and antagonist of benzodiazepines. J. Med. Chem. **25**:337–339, 1982.