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Central Excitatory Actions of Flurazepam

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ROSENBERG, H. C. *Central excitatory actions of flurazepam.* PHARMACOL. BIOCHEM. BEHAV. 13(3) 415–420, 1980.—Toxic actions of flurazepam (FZP) were studied in cats, mice and rats. High doses caused an apparent central excitation, most clearly seen as clonic convulsions, superimposed on general depression. Following a lethal dose, death was always associated with convulsions. Comparing the relative sensitivity to central depression and excitation revealed that rats were least likely to have convulsions at doses that did not first cause loss of consciousness, while cats most clearly showed marked central excitatory actions. Signs of FZP toxicity in cats included excessive salivation, extreme apprehensive behavior, retching, muscle tremors and convulsions. An interaction between FZP and pentylenetetrazol (PTZ) was shown by pretreating mice with FZP before PTZ challenge. As a function of dose, FZP first protected against convulsions and death. At higher doses, however, convulsions again emerged. These doses of FZP were lower than those that would alone cause convulsions. These results may be relevant to the use of FZP in clinical situations in which there is increased neural excitabilty, such as epilepsy or sedative-hypnotic drug withdrawal.

Flurazepam Benzodiazepine Central nervous system stimulation Drug overdose Drug abuse

BENZODIAZEPINES are the most widely prescribed psychoactive drugs. Unlike most other members of this group, which are primarily used to treat anxiety, flurazepam (FZP) has enjoyed great success as a hypnotic. Early descriptions of FZP pharmacology [14,15] indicated central nervous system actions much like those of diazepam, chlordiazepoxide and other benzodiazepines. The major difference noted was that, compared to chlordiazepoxide, FZP seemed a more potent central depressant, but less potent in its ability to block conditioned avoidance behavior [15]. This may be related to FZP's use as a hypnotic rather than as an antianxiety drug, although definitive clinical proof of a major difference between FZP and other benzodiazepines has not been forthcoming (cf. [7]). In studies of specific high-affinity benzodiazepine binding, FZP displaced the bound radiolabelled diazepam from cerebral cortical membranes, and its potency was as expected if it acts at this specific binding site [2,12]. Furthermore, chronic high-dose FZP treatment causes a compensatory decrease in the number of these specific binding sites [3,16]. Overall, it appears that FZP probably acts much as do other benzodiazepines. There are, however, two indications in the experimental literature that FZP, at least at high doses, may have a central excitatory action not described for other benzodiazepines.

In a major review comparing different actions of benzodiazepines in various species [14], it was reported that high doses of FZP (100 mg/kg, PO) caused cats to convulse, and death after a lethal dose (400 mg/kg) was the result of convulsion rather than central depression. This observation was in sharp contrast to the toxic actions of FZP in other species (primarily mice and rats), and to the actions of other benzodiazepines in all species tested, including cats. In an earlier study [15], it was noted that 2 out of 8 dogs being maintained on 40 mg/kg FZP daily died during convulsions after several weeks of treatment. It is not clear whether this is a manifestation of the same drug action reported in cats, or a different toxicity. Because this ability of FZP to cause convulsions is unique among the clinically useful benzodiazepines, it may indicate a fundamental difference in its pharmacology. Experiments were performed to describe quantitatively the effects of FZP, with emphasis on the acute toxic actions resulting from high doses.

METHOD

Mouse Experiments

Male ICR mice, 20–25 g, were used. Six different doses of FZP ranging from 20 to 600 mg/kg were injected IP. Each was tested in a group of 10 mice. Drug solutions and a saline control solution were coded so that observers were unaware of the dose being administered. Each animal was assigned a rating for degree of ataxia (0=none, 1=slight, 2=gross ataxia, 3=unable to walk at all), and for ability to remain on a standard vertical screen for at least one minute (+or 0). (The screen was a 44×22 cm rectangle of 18 ga aluminum wire arranged in 13 mm squares.) Loss of righting response, convulsive activity, and any other signs of drug action were recorded. In a second experiment, groups of 5 mice were injected with 125 mg/kg pentylenetetrazol (PTZ), IP, to produce convulsions. Five minutes before PTZ, mice were pretreated with an IP injection of FZP (2 to 200 mg/kg), diazepam (0.5 to 50 mg/kg), physiologic saline, or diazepam solvent.

Rat Experiments

Male Sprague-Dawley rats, 300–400 g, were given 4 different doses of FZP (20, 40, 100 and 200 mg/kg) by IP injection. Each dose was tested in 5 rats. Animals were assigned a rating for the degree of ataxia produced, as described above. Loss of righting response, convulsive activity and other signs of drug action were recorded.

Cat Experiments

Adult mongrel cats of both sexes were given FZP, 2 to 100

TABLE 1
RATING SCALE FOR FLURAZEPAM ACTIONS IN CATS

Neurological parameter	Ratings
Level of consciousness	3=awake, or easily aroused by noise 2=only aroused by loud, repeated noise 1=aroused when touched or moved, not by noise 0=unarousable
Muscle tone	3=normal 2=decreased in hindlegs, but not flaccid 1=decreased in front, back legs flaccid 0=gross loss of tone in all 4 legs
Standing	3=normal 2=stands with difficulty, falls 1=front legs support body, but not back 0=unable to support self
Walking	3=normal gait 2=moderate ataxia, does not fall 1=grossly ataxic, falls 0=unable to walk at all
Land from right side up position	3=normal 2=heavy landing. Legs support weight poorly 1=stays upright, but unable to support weight 0=unable to support weight, falls to side
Land from upside down position	3=normal. Lands lightly on all 4 feet 2=slowed response 1=minimal reflex response 0=no response

mg/kg, IP. Each dose was tested in 5–9 animals. In most trials the observer was not aware of the dose being given. Animals were observed continuously for several hours, and drug effects were recorded on a predetermined schedule as long as required to determine the total duration of drug action. Some signs of drug action were subjectively evaluated on a scale of 0 to 3 in a manner similar to that previously described for recording depressant drug actions in cats [13]. Those signs of drug-induced neurological deficit that were rated are presented in Table 1. Muscle tone was evaluated by direct palpation while suspending the animal by the scruff of the neck. For the two tests requiring the animals to be dropped, a thick foam rubber pad was used, the animals were dropped from a height of 1 m.

Drugs

Flurazepam dihydrochloride was dissolved in distilled water. The pH was adjusted to 5.8–6.2 with NaOH and the final volume adjusted to make the 100 mg/ml stock solution. This was used as such, or diluted with physiologic saline so that the dose could be administered in a volume of 1 ml/kg for cats and rats, and 10 ml/kg for mice. In experiments with rats requiring higher doses, a 200 mg/ml solution was made. Diazepam was prepared in a solvent consisting of 50% water, 10% ethanol and 40% propylene glycol. The final concentration was such that mice received the dose in a 1 or 2 ml/kg volume, thus minimizing the amount of solvent injected. For the more concentrated solutions, dilute HCl was added as required to achieve solubilization. Final pH of diazepam solutions ranged from 2.5 to 6.5. Pentylenetetrazol was prepared as a 12.5 mg/ml solution in physiologic saline. Mice received 10 ml/kg. Flurazepam and diazepam were generously supplied by Hoffmann-LaRoche, Inc.

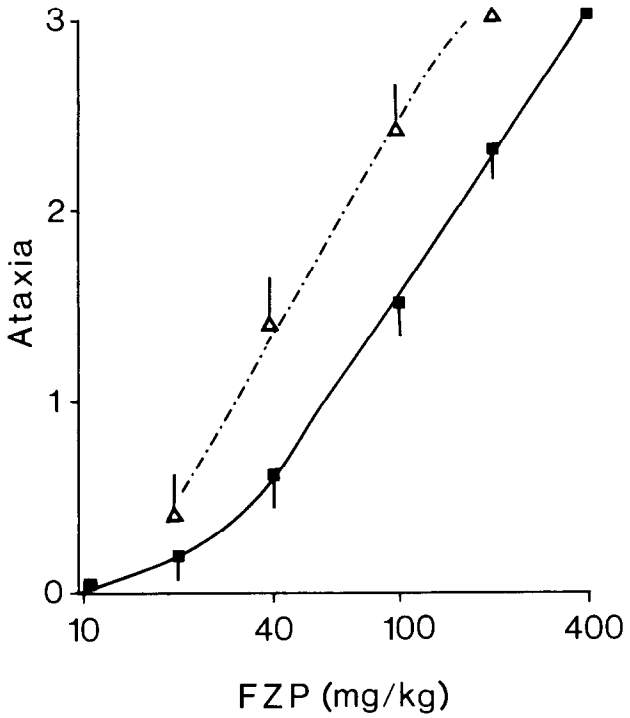

FIG. 1. Mean ataxia ratings at peak effect as a function of FZP dose in mice and rats. Each value is the mean of ratings from 5 rats (△) or 10 mice (■).

Statistical Analyses

Statistical tests used in analysis of the data are indicated in the RESULTS. In all cases, a probability less than 0.05 was the accepted level of significance. Where graded responses are summarized in figures, vertical lines are used to indicate the standard error of the mean; where none is shown, the SE was smaller than the symbol used to plot the point.

RESULTS

Dose-Response in Mice

Increasing doses of FZP caused increasing impairment of motor ability as measured by degree of ataxia (Fig. 1) and ability to remain on the vertical screen (Fig. 2). Onset of drug activity was 1–3 min after injection, and peak action was 5–10 min after injection. No mice receiving 200 mg/kg or less lost the righting response. Of the 10 mice that were injected with 400 mg/kg, half lost the righting response before onset of convulsive activity. In the other half, seizures clearly began before loss of righting response. All mice dosed with 400 mg/kg displayed convulsions (Fig. 2). This was primarily seen as clonic activity involving all 4 limbs. Four mice in this group also had episodes of tonic extension, and 9 of the 10 died following continued clonus, 5–12 min after injection. All 10 mice injected with 600 mg/kg also had convulsions. Six

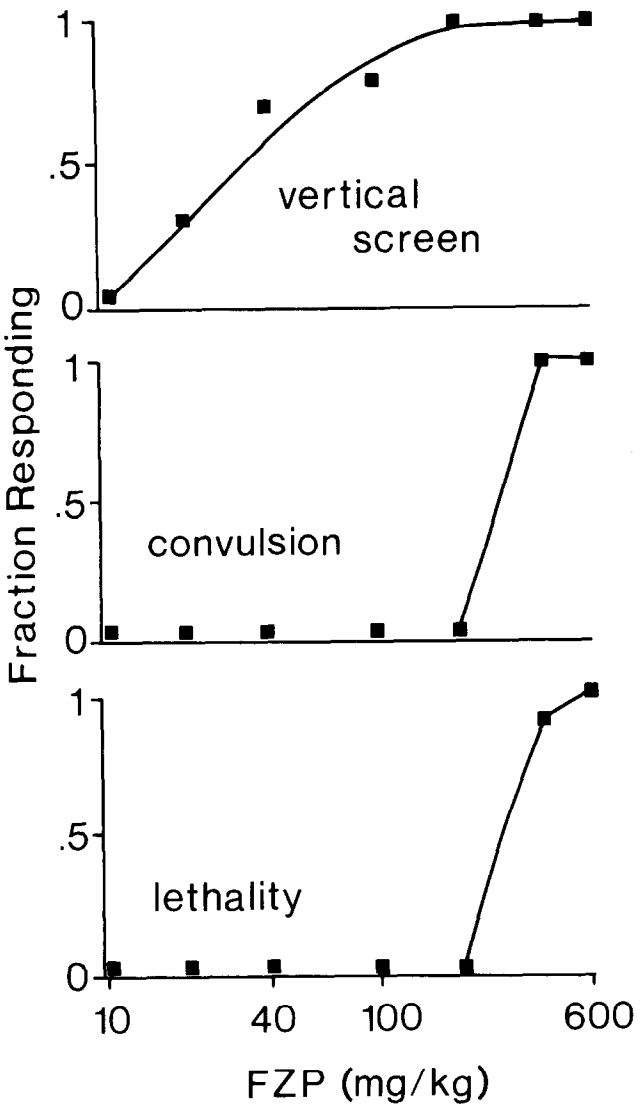

FIG. 2. Effect of FZP on motor weakness (measured by ability to stay on a standard vertical screen), convulsions and lethality. Ten mice were tested at each dose.

mice had seizures with both tonic and clonic components, one had tonic-clonic convulsions and a tonic extensor convulsion, and 3 had only clonic movements. In 8 of these 10 mice, convulsive activity began when animals still had an intact righting response. Even larger doses of 1–2 mg/kg were found to cause a more sudden onset of convulsions, with more prominent tonic extension.

Dose-Response in Rats

The effect of FZP in rats was similar to that in mice, although there were differences in sensitivity to the drug between the two species. Rats were more easily rendered ataxic than were mice (Fig. 1). None of the rats lost the righting response after 100 mg/kg, but 3 out of 5 did so after 200 mg/kg. Rats seemed relatively less sensitive to the convulsant action of FZP. Of the 5 rats that received 200 mg/kg, only the 3 that lost the righting response had clonic convulsions, and only after they had lost consciousness. One rat

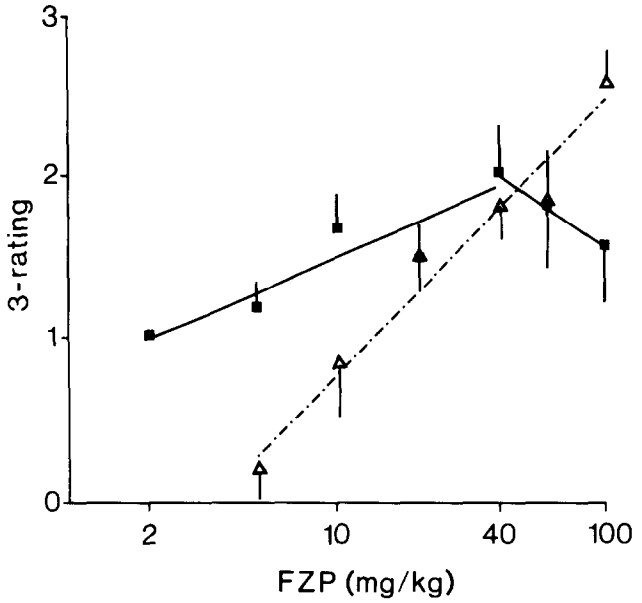

FIG. 3. Peak FZP effect on walking ability (△) and on muscle tone (■) in cats. Values on the ordinate are the differences between the maximum possible rating (3) and the actual rating assigned, as outlined in Table 1. Each value represents the mean of values derived from 5–7 observations.

died 12 min after injection; the others regained consciousness about 16 min after injection.

Dose-Response in Cats

After IP administration of FZP, drug effects were first noticed within 3–5 min. Peak drug effect occurred 10–40 min after injection, after which recovery progressed rapidly for about 2 hours, then much more slowly. Following doses of 2 or 5 mg/kg, recovery was complete in about 12 hours. Much more time was required for recovery after larger doses. The longest duration of action was after 60 mg/kg, when approximately 96 hours was needed for full recovery. Some animals receiving 100 mg/kg recovered more rapidly than those that had been given 60 mg/kg. However, the overall difference was slight and the ratings of the two groups were not significantly different ($p>0.05$ by t test).

Most of those FZP actions specifically evaluated (Table 1) increased as the dose increased from 2 to 100 mg/kg. Thus, ability to maintain posture when dropped right-side up, and ability to land normally on all 4 feet after being dropped upside-down were impaired much as was walking ability, which is shown in Fig. 3. Standing ability was similarly affected, except that this was a much less sensitive indicator of drug action since 40 mg/kg or more was required to cause any measurable effect. As grossly measured on the 0 to 3 scale outlined in Table 1, FZP rarely decreased the apparent level of consciousness. In fact, the only times when decreased consciousness was regularly observed was during or immediately after a seizure (described below).

The cat proved to be a very sensitive indicator of the behavioral disinhibition that is a well-known action of benzodiazepines (reviewed in reference [6]). Within a few minutes of FZP injection animals began to eat voraciously. This effect has been reported to occur in cats given small doses of oxazepam, diazepam, N-methyl-lorazepam, medazepam and

TABLE 2
SIGNS OF CENTRAL STIMULATION IN CATS TREATED WITH FZP

Sign	Incidence after:	
	60 mg/kg	100 mg/kg
Piloerection	0/6	1/9
Retching	2/6	2/9
Muscle twitches or tremors	1/6	2/9
Salivation	0/6	6/9
Abnormal apprehension	4/6	8/9
Clonic convulsions	0/6	6/9
Extensor posturing, jerking	0/6	7/9

chlordiazepoxide [11]. After doses of 40, 60 or 100 mg/kg, central depression or convulsions intervened. At lower doses, however, animals continued to eat excessively for several hours, often vomiting large amounts of partially digested food. Excessive food intake was reflected in a significant increase in body weight over a 24 hour period following dosing. The largest average weight gain was 6% (range 3–12%) in the group treated with 5 mg/kg. Statistically significant (by paired t test) 24 hour weight gains were recorded after 2, 5, 10, 20 and 100 mg/kg, but not after 40 or 60 mg/kg doses. Another indication of behavioral disinhibition was an apparent magnification of the animal's pre-drug behavior. The cats used in this study generally appeared either moderately apprehensive or else "friendly" and playful. After FZP, animals that had been apprehensive became more so, were difficult to handle, and tried to escape at every opportunity. The friendly animals became very affectionate, often crying loudly until the observer handled them. After the highest doses (60 and 100 mg/kg), most animals became extremely apprehensive irregardless of their pre-drug behavior (Table 2).

Several signs of FZP toxicity that are probably indicative of central stimulation are listed in Table 2. Their appearance was dose related, and none was seen at doses of 40 mg/kg or less. Another manifestation of FZP action only evident with large doses was an apparent reversal of muscle relaxation. Muscle tone after 60 or 100 mg/kg was highly variable but, overall, was greater than after lower doses. In a few animals, muscle tone was actually increased compared to pre-drug control. The dose-response relationship describing muscle relaxation as a function of FZP dose was biphasic, with peak drug effect at 40 mg/kg (Fig. 3). To test the validity of this observation, the slope of the calculated regression line ($y = m \log x + b$) for data from 2 to 40 mg/kg was compared to that for data from 40 to 100 mg/kg. For the lower portion of the curve, the slope (and 95% confidence interval) for the calculated regression line was 0.714 (0.281 to 1.147). For the upper portion of the curve, the slope (and confidence interval) was −2.254 (−2.728 to −1.780). Since the slopes are opposite in sign and neither one of the confidence intervals includes 0, it is highly probable that this is a true biphasic relationship.

The most dramatic evidence of FZP toxicity was the occurrence of convulsions. After 100 mg/kg FZP, 7 of 9 animals had convulsive activity (Table 2). Before onset of seizure activity, animals usually first displayed signs of central depression (Table 1) upon which evidence of central stimulation was then superimposed. Animals often seemed less aware of their surroundings, but none lost consciousness before seizure activity began. The 7 animals having seizures each had from 2 to 6 episodes, each of which was of 10–30 sec duration. None of the animals died. The seizures took two forms, and both types were seen in 6 of the subjects. Six animals had clear-cut major motor seizures with clonic activity involving all 4 limbs, trunk, neck and face muscles. Some were bilaterally synchronous, while others were manifested as stepping movements accompanied by extension of the head. On one occasion the seizure rapidly progressed to tonic extension, followed by clonus. Clonic seizures began 10–30 min after FZP injection. During the other type of seizure activity, animals displayed tonic extensor posturing of the legs and head, often preceded by or intermixed with bilaterally synchronous jerking. Animals would fall in this posture, sometimes vocalizing loudly as if delerious, and were unresponsive to all stimuli. These seizures began 7–35 min after FZP injection. Animals were rarely fully conscious between seizures. Typically, they would cry repeatedly and move their heads as if trying to right themselves. At this time they were only poorly responsive to visual or auditory stimuli. Handling the animal would frequently precipitate another bout of tonic extension and jerking. An additional 4 cats were dosed with 150 mg/kg FZP. All had both forms of seizure activity starting 10–20 min after dosing. None lost consciousness before onset of seizures.

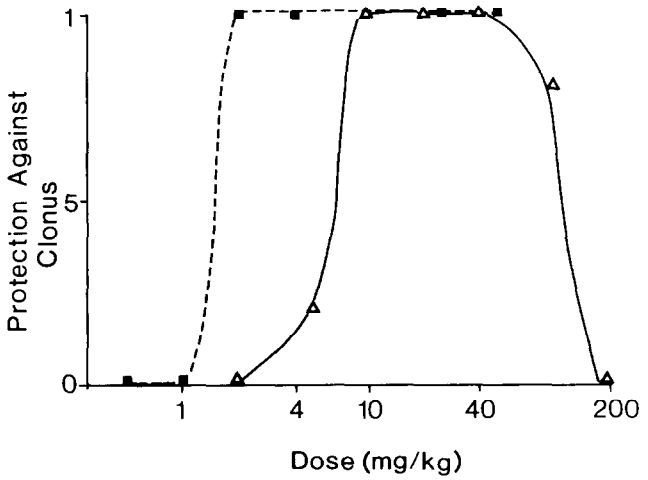

FIG. 4. Interaction between benzodiazepines and PTZ. Each group of 5 mice was pretreated with a dose of diazepam (■) or FZP (△) 5 min before challenge with 125 mg/kg PTZ, IP.

Two animals were given 40 mg/kg FZP by intravenous infusion. One received the dose over 4 min, the other over 1 min. (More rapid injection resulted in rapid death, apparently from respiratory arrest). Both cats had clonic convulsive activity starting before the end of the infusion, followed by several more seizure episodes.

FZP-PTZ Interaction

All doses of FZP protected against the tonic extensor component of PTZ-induced convulsions. Clonic convulsions were completely blocked by 10, 20 and 40 mg/kg. However, clonic convulsions occurred in 1 of 5 mice treated with 100 mg/kg, and in all 5 treated with 200 mg/kg FZP (Fig. 4). Three of these mice had prolonged clonus and died 11–12 min after

PTZ injection. This is in contrast to the effects of FZP alone, which caused neither convulsions nor death except at doses higher than these (Fig. 2). All doses of diazepam protected against tonic extension, and all except the lowest protected against clonus as well. An average ataxia rating of 2.2 was measured in mice treated with 25 mg/kg diazepam. This is comparable to the ataxia produced by 200 mg/kg FZP (Fig. 1). All mice pretreated with saline or diazepam solvent died in characteristic tonic extensor convulsions after PTZ injection.

DISCUSSION

The data show that as the dose of FZP is increased to toxic levels, central stimulation is superimposed on the depression. Thus, in contrast to other benzodiazepines, FZP overdose is associated with signs of central excitation, including convulsions. This was seen in cats, as has been previously noted [14], and in rats and mice. Previous reports [14,15] did not indicate this action of FZP in rodents. A possible explanation may be the differing routes of administration. In the earlier studies, oral drug administration was most often used. This would maximize the first-pass metabolic effect, which is apparently very great in man [10], in dog [4], and probably in other species as well. Assuming that FZP, and not a metabolite is responsible for the central stimulation, any factor favoring rapid metabolism would limit the chances of observing the central stimulation. Using oral administration, as has been most frequently done, does not favor high peak FZP levels and allows for more extensive metabolism to inactive products and to active products that might mask excitation. Based on the rapid onset of convulsions following large doses of FZP, especially after IV administration, it seems likely that unmetabolized FZP, rather than one of its metabolites, is responsible for this action.

Although no other clinically useful benzodiazepine has been reported to cause convulsions, an experimental drug, R05-3663, is a convulsant benzodiazepine which apparently interferes with GABA-mediated inhibition [17]. While it possesses the benzodiazepine ring structure, R05-3663 lacks the 5-phenyl substitution and the electronegative group at position 7 present on all clinically useful benzodiazepines. It also lacks an N-1 substitution, where FZP has a rather large diethyl-aminoethyl group. Unlike FZP, R05-3663 does not appear to bind to the benzodiazepine receptor [18]. It seems unlikely that the neuropharmacological mechanism(s) responsible for most FZP actions are like those of R05-3663, yet it is possible that high concentrations of FZP may also block GABA-mediated inhibitory transmission. Future experiments will explore this possibility.

The evidence indicates that there is a species difference in the relative susceptibility to depressant and excitatory actions of FZP. It seems, however, that this is a quantitative rather than a qualitative difference. It may be that, in man, overdose with FZP might present as excitation, depression, or a combination of both as was clearly seen in the experiments with cats. Recognition of such a situation would be crucial to proper treatment. It seems unlikely that central excitation from FZP would be encountered in the vast majority of cases. However, there are circumstances that may predispose to this effect, either by favoring high tissue levels of unmetabolized drug, or by somehow rendering the nervous system more excitable. One factor could be intravenous abuse, which would produce very high peak levels of FZP. The results of this study indicate that, in the cat, IV FZP does cause convulsions at lower doses than required by the IP route, but a rather large dose is still required to produce this effect. In one trial [5], up to 1.5 mg/kg FZP was given intravenously to surgical patients. No signs of central stimulation were reported. Whether higher, or more rapidly injected doses might cause convulsive activity is open to question. At least it does not appear that man is unexpectedly more sensitive than other species to central stimulation by FZP.

Epileptics may also be more likely to experience central stimulation rather than depression, especially if not taking medication or not adequately controlled. One report [1] does describe a patient suffering atypical seizures that first presented as "non-REM nightmares", and which were eventually controlled by phenytoin. Before correct diagnosis was made, the patient was first treated with diazepam (up to 30 mg daily), and the number of attacks rose from about 15 to about 20 each day. He was then treated with FZP (up to 50 mg daily), and the rate of attacks increased to about 2 each hour. This may be an example of supersensitivity to FZP excitation in an epileptic patient. The interaction between PTZ and FZP (Fig. 4) may be relevant to this possibility, since in both cases the nervous system has been rendered hyperexcitable, and more sensitive to chemical stimulation.

Another possibility that should be considered is that of FZP actions in persons tolerant to or dependent on ethanol, barbiturates or other depressant drugs. Such individuals may be at least partially cross-tolerant to FZP's depressant action, but may well be supersensitive to the drug's excitant properties. Several studies [8, 9, 19] have shown that animals dependent on sedative-hypnotics or ethanol are much more sensitive to chemical convulsants. FZP treatment of a person dependent on a depressant drug might then cause a worsening rather than an improvement.

Finally, it should be noted that the central stimulation produced by large doses of FZP indicates that this drug has an action that is different from diazepam, chlordiazepoxide and other clinically used benzodiazepines. It will be of interest to determine the nature of this difference and how it might influence use or abuse of FZP.

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