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The β-Carboline Derivatives ZK 93426 and FG 7142 Fail to Precipitate Abstinence Signs in Diazepam-Dependent Cats

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GIORGI, O., M. G. CORDA, A. FERNANDEZ AND G. BIGGIO. *The β-carboline derivatives ZK 93426 and FG 7142 fail to precipitate abstinence signs in diazepam-dependent cats.* PHARMACOL BIOCHEM BEHAV **32**(3) 671–675, 1989.—The aim of the present study was to investigate the ability of different benzodiazepine recognition site antagonists (Ro 15-1788 and ZK 93426) and inverse agonists (Ro 15-4513, FG 7142 and CGS 8216) to induce abstinence signs in diazepam-dependent cats. Different groups of cats were challenged with each of the benzodiazepine recognition site ligands under investigation 24 hours after the last dose of chronic treatment with diazepam (7 mg/kg, IP at 8.00 a.m. and 8.00 p.m. for 21 consecutive days). The benzodiazepine derivatives Ro 15-4513 and Ro 15-1788 precipitated an abstinence syndrome within minutes after IP administration. The pyrazoloquinoline derivative CGS 8216 also induced withdrawal signs that were less severe and had a longer latency than those elicited by Ro 15-4513 and Ro 15-1788. Abstinence signs included tremors, increased muscle tone, irritability, fear, arched-back posture, pupillary dilation and vocalizations. On the other hand, the β-carboline derivatives ZK 93426 and FG 7142 failed to precipitate abstinence signs in diazepam-dependent cats when given at doses that prevented the acute effects of diazepam. Our results demonstrate that the ability to induce withdrawal signs in diazepam-dependent cats depends on the chemical structure of the challenge drug (i.e., benzodiazepine or pyrazoloquinoline), since β-carboline antagonists like ZK 93426 and partial inverse agonists like FG 7142 lack this property.

Benzodiazepine receptor antagonists ZK 93426 Ro 15-1788 Ro 15-4513 CGS 8216 FG 7142 Diazepam Abstinence syndrome Cat

BENZODIAZEPINES (BZDs) exert their pharmacological effects via interaction with specific recognition sites which are part of the macromolecular GABA receptor complex (7,10). Experimental evidence indicates that BZD recognition site ligands can be considered as a continuum of three overlapping groups, conventionally designated agonists, antagonists and inverse agonists, according to their modulatory effects on the GABAergic transmission (4). BZD recognition site agonists, like diazepam, exert a positive modulatory action on the GABA receptor complex that mediates the anxiolytic and anticonvulsant effects of these agents (1, 4, 7, 10, 24). In contrast, inverse agonists like the β-carboline derivative FG 7142, the imidazobenzodiazepine derivative Ro 15-4513 and the pyrazoloquinoline CGS 8216, have negative efficacy on the GABAergic transmission and display anxiogenic and proconvulsant actions (1, 2, 4, 5, 9, 13, 24, 26). In between these two groups, BZD recognition site antagonists, such as the BZD derivative Ro 15-1788 and the β-carboline derivative ZK 93426, are devoid of intrinsic effects but prevent the actions of both agonists and inverse agonists (4, 12, 13).

Clinical studies have shown that the anticonvulsant, hypnotic and anxiolytic effects of BZD recognition site agonists may be altered by tolerance, and physical dependence may be detected by the appearance of an abstinence syndrome upon the abrupt cessation of drug administration. In its most severe form, the abstinence syndrome is characterized by insomnia, tremors, anorexia, autonomic symptoms and, less frequently, major motor seizures (14, 16, 23, 28, 30). Accordingly, the rapid displacement of BZD agonists from their binding sites induced by the acute administration of Ro 15-1788 or CGS 8216 precipitates an acute abstinence syndrome in different animal species when given after a long-term treatment with anxiolytic BZDs (8, 17, 19, 20, 29).

In contrast, it has been recently reported that the inverse agonist FG 7142 does not precipitate withdrawal signs in diazepam-dependent cats, although it can reverse the acute effects of

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diazepam (25). This peculiar property of FG 7142 has been attributed to the fact that β-carbolines interact with domains on the macromolecular GABA receptor complex which are partly different from those recognized by BZD derivatives (25). Alternatively, the failure of FG 7142 to elicit withdrawal signs in diazepam-dependent cats may be related to the inverse agonist properties of this drug (4,25).

In order to test these two possibilities we examined the ability of different BZD recognition site antagonists (Ro 15-1788 and ZK 93426) and inverse agonists (Ro 15-4513, FG 7142 and CGS 8216), to precipitate a BZD abstinence syndrome in diazepam-dependent cats.

Here we report that while Ro 15-4513, Ro 15-1788 and CGS 8216 induce an abstinence syndrome in cats chronically treated with diazepam, ZK 93426 and FG 7142 fail to do so. It is proposed that the ability to elicit withdrawal signs depends on the chemical structure of the challenge drug since β-carboline antagonists and inverse agonists lack this property.

METHOD

Subjects

Adult mongrel cats, whose body weights ranged from 3.2 to 4.1 kg in males (n = 10) and 2.1 to 3.2 kg in females (n = 8), were used in these studies. Animals were individually housed and had free access to food and water. Cats had no prior history of exposure to neuropharmacological agents.

Drug Treatments and Behavioral Evaluations

Acute treatment. Cats were kept in the animal-house for a 10-day habituation period prior to the experiments.

The acute effects of Ro 15-1788, ZK 93426, Ro 15-4513, FG 7142 and CGS 8216 were evaluated in two independent test sessions that preceded the chronic treatment with diazepam.

In the first test session, different groups of cats were injected IP with 10 mg/kg of Ro 15-1788, Ro 15-4513, ZK 93426, FG 7142, or CGS 8216. Cats were observed continuously for 90 min after drug injection and the behavioral effects (i.e., tremors, increased muscle tone, irritability and vocalizations) were recorded.

The second test was carried out one week later. Cats were injected with vehicle or with 10 mg/kg, IP of each drug 10 min before diazepam at the dose of 7 mg/kg, IP. The ability of Ro 15-1788, Ro 15-4513, ZK 93426, FG 7142 and CGS 8216 to antagonize the motor incoordination, loss of righting reflex and sleep induced by diazepam was evaluated continuously for 2 hr following diazepam injection.

Chronic treatment. After a drug-free period of one week, cats were treated with diazepam given in two equivalent IP doses of 7 mg/kg at 8 a.m. and 8 p.m. on each of 21 days. Diazepam was given as the commercially available injectable preparation (Valium, Hoffmann-La Roche, Basel, Switzerland). Cats were observed daily from 8 a.m. to 10 a.m. and the effects of diazepam (ataxia, loss of righting reflex, sleep) were rated as present or absent in a series of 12 consecutive 10 min intervals throughout the total period of observation.

Twenty-four hours after the last diazepam dose, cats were treated with either Ro 15-1788, Ro 15-4513, ZK 93426, FG 7142 or CGS 8216 at the doses used in the previous test session. Cats were observed by two raters unaware of the animal treatments, beginning immediately after drug injections. All abnormal motor, autonomic and behavioral activity was recorded using a check list to determine the prevalence of the individual withdrawal signs. The abstinence signs taken into account were tremors of the head and of the body and limbs, movement-induced tremor, increased muscle tone, arched-back posture, decreased motor activity, irritability, aggressiveness or fear, piloerection, pupillary dilation, increased respiratory rate and panting (i.e., mouth open and tongue extended), vocalizations, salivation and defecation. These signs were rated as present or absent in a series of 12 consecutive intervals 15 min in duration uniformly distributed throughout a 6-hr period of observation.

TABLE 1

DIAZEPAM-INDUCED MOTOR INCOORDINATION: PREVENTION BY BENZODIAZEPINE RECOGNITION SITE ANTAGONISTS AND INVERSE AGONISTS

Pretreatment	Onset of Motor Incoordination (min)
Vehicle	7 ± 2
Ro 15-1788	38 ± 2*
ZK 93426	33 ± 3*
Ro 15-4513	45 ± 5*
CGS 8216	42 ± 7*
FG 7142	32 ± 4*

Cats were pretreated with vehicle or one of the listed drugs at the dose of 10 mg/kg, IP, 10 min before diazepam (7 mg/kg, IP). Following the administration of diazepam, the animals were observed continuously for two hours and the state of motor coordination was recorded at 5-min intervals.

Results are the mean ± S.E.M. of 3 cats per group.

*$p<0.001$ vs. the vehicle-pretreated group.

Drugs

ZK 93426 (ethyl-5-isopropoxy-4-methyl-β-carboline-3-carboxylate) and FG7142 (methylamide-β-carboline-3-carboxylate) were kindly provided by Ferrosan, Copenhagen, Denmark and by Schering A.G., Berlin, FRG. Diazepam (Valium), Ro 15-1788 (ethyl-8-fluoro-5·6-dihydro-5-methyl-6-oxo-4H-imidazo-[1.5 a] [1.4] benzodiazepine-3-carboxylate) and Ro 15-4513 (ethyl-8-azido-5·6-dihydro-5-methyl-6-oxo-4H-imidazo-[1.5a] [1.4] benzodiazepine-3-carboxylate) were gifts from Hoffmann-La Roche, Basel, Switzerland and CGS 8216 (2-phenyl-pyrazolo-4·5-c-quinolin-3-[5H]-one) was kindly provided by Ciba-Geigy, Summit, NJ. All the above drugs (except diazepam) were suspended in distilled water with 50 μl of Tween 80 per 5 ml.

RESULTS

Acute Treatments

The acute administration of 10 mg/kg, IP, of Ro 15-1788, Ro 15-4513, CGS 8216 or ZK 93426 failed to induce any relevant behavioral effect. On the other hand, the administration of FG 7142 (10 mg/kg, IP) induced tremors, rigidity and irritability in 2 out of 3 cats. The behavioral effects of FG 7142 had a rapid onset (less than 10 min) and persisted for 30 to 40 min (not shown).

One week later, different groups of cats were treated with vehicle or with one of the tested drugs at the dose of 10 mg/kg, IP, 10 min before diazepam (7 mg/kg, IP). As shown in Table 1, cats treated with diazepam alone displayed severe motor incoordination associated with reduced muscle tone and sedation within less than 10 min. When cats were pretreated with 10 mg/kg of either Ro 15-1788, ZK 93426, Ro 15-4513, CGS 8216 or FG 7142, the onset of diazepam-induced motor incoordination was delayed by at least 30 min (Table 1). All these compounds were also able to

TABLE 2

FAILURE OF ZK 93426 TO PRECIPITATE ABSTINENCE SIGNS IN CATS CHRONICALLY TREATED WITH DIAZEPAM

Withdrawal Signs	Ro 15-1788 (n = 3)	ZK 93426 (n = 3)	Ro 15-1788 24 hr After ZK 93426 (n = 3)	ZK 93426 24 hr After Ro 15-1788 (n = 3)
1. Tremors	3	0	3	0
2. Increased muscle tone	3	0	2	0
3. Arched-back posture	3	0	2	0
4. Decreased motor activity	3	0	3	0
5. Irritability-fear	3	0	3	0
6. Piloerection	3	0	3	0
7. Pupillary dilatation	3	0	2	0
8. Panting	2	0	2	0
9. Vocalization	3	0	3	0
10. Profuse salivation	3	0	3	0
11. Defecation	2	0	1	0

Cats were treated with diazepam (7 mg/kg, IP, twice daily at 8 a.m. and 8 p.m.) for 21 consecutive days. Twenty-four hr after the last injection of diazepam, a challenge dose of Ro 15-1788 or ZK 93426 was administered IP and cats were observed for 6 hr thereafter. The listed values indicate the number of cats displaying each behavioral sign. Cats that displayed no withdrawal signs after ZK 93426 were treated 24 hr later with Ro 15-1788. Animals challenged with Ro 15-1788 in the first place were injected 24 hours later with ZK 93426. Ro 15-1788 and ZK 93426 (10 mg/kg, IP) were suspended in distilled water with 50 μl of Tween 80/5 ml.

TABLE 3

BEHAVIORAL EFFECTS OF BENZODIAZEPINE RECOGNITION SITE INVERSE AGONISTS IN DIAZEPAM-DEPENDENT CATS

Withdrawal Signs	Ro 15-4513 10 mg/kg (n = 3)	FG 7142 10 mg/kg (n = 3)	CGS 8216 10 mg/kg (n = 3)
1. Tremors	2	1	0
2. Increased muslce tone	2	1	0
3. Arched-back posture	3	0	0
4. Decreased motor activity	3	0	3
5. Irritability-fear	3	1	3
6. Piloerection	3	0	0
7. Pupillary dilatation	3	0	3
8. Panting	2	0	0
9. Vocalization	3	0	3
10. Profuse salivation	3	0	3
11. Defecation	1	0	2

Cats were treated with diazepam (7 mg/kg, IP, twice daily at 8 a.m. and 8 p.m.) for 21 consecutive days. Twenty-four hr after the last injection of diazepam, a challenge dose of one of the tested drugs was administered IP and cats were observed for 6 hr thereafter. The listed values indicate the number of cats displaying each behavioral sign.

decrease the severity of diazepam-induced ataxia (not shown). Moreover, tremors and rigidity elicited by FG 7142 disappeared within 15 min after diazepam administration. No withdrawal signs were observed after the combined acute administration of diazepam plus the various antagonists and inverse agonists.

These results indicate that, at the doses used, the compounds tested reach sufficiently high cerebral concentrations in order to compete with diazepam (7 mg/kg, IP) for BZD recognition sites.

Chronic Treatment With Diazepam

The first doses of diazepam during the long-term treatment elicited clear-cut signs of sedation, decreased muscular tone and ataxia. All these signs became apparent within 5 to 10 min and were still present 2 hours later. Tolerance rapidly developed to these effects, so that diazepam-induced sedation and ataxia were markedly decreased by the fifth day of treatment and there were no observable effects of diazepam after 15 days of treatment. No stimulatory effects or withdrawal signs were observed throughout the 3 weeks of chronic treatment with diazepam.

BZD Receptor Ligand-Induced Abstinence Syndrome

Cats were challenged with different BZD recognition site ligands 24 hours after completing the 21-day diazepam treatment. In agreement with previous reports, all three cats treated with Ro 15-1788 (10 mg/kg, IP) displayed an abstinence syndrome within 5 min after the administration of the drug. The prevalence of the abstinence signs is shown in Table 2. If left undisturbed, cats stayed crouched in their cages or moved around very little. Two cats became aggressive if handled, whereas the third one behaved as if frightened and rapidly fled back to its cage when removed from it. Piloerection, pupillary dilatation, profuse salivation, and repeated vocalizations were present in all animals. Tremors of the body and limbs, increased muscle tone, increased respiratory rate, defecation and panting were also seen. These signs persisted for about 2 hours.

In contrast, none of the abstinence signs or any other evidence of drug action were seen in the cats challenged with the β-carboline antagonist ZK 93426 (Table 2). Moreover, 24 hours later the same animals treated with ZK 93426 or Ro 15-1788 were again challenged with 10 mg/kg of Ro 15-1788 or ZK 93426, respectively. The second treatment with Ro 15-1788 elicited an abstinence syndrome similar to that observed in the group of cats challenged with this drug in the first place. On the contrary, the administration of ZK 93426 to cats previously challenged with Ro 15-1788 still failed to precipitate the abstinence syndrome (Table 2).

Similarly, the β-carboline inverse agonist FG 7142 failed to induce an abstinence syndrome in diazepam-dependent cats. Thus, as shown in Table 3, only one out of three cats challenged with FG 7142 showed tremors, increased muscle tone and fear of very short duration (10 to 15 min). In addition, no clear-cut abstinence signs were elicited by FG 7142 in doses up to 20 mg/kg, IP (not shown).

On the other hand, the partial inverse agonist BZD Ro 15-4513 (10 mg/kg, IP) precipitated abstinence signs whose intensity and time course were similar to those elicited by Ro 15-1788. Thus, arched-back posture, decreased motor activity, irritability, piloerection, pupillary dilation, vocalization and profuse salivation were observed in all three animals challenged with Ro 15-4513 and two out of three cats also displayed tremors, increased muscle tone and panting. These signs had a rapid onset (less than five min) and persisted for more than two hours (Table 3). Finally, CGS 8216 (10 mg/kg, IP) also induced an abstinence syndrome, although the onset of the abstinence signs was slower with this compound than with Ro 15-1788 and Ro 15-4513. Thus, aggressiveness and pupillary dilatation appeared in all three cats treated with this drug with a latency of at least 15 min. Vocalizations, profuse salivation and defecation were also observed 25–30 min

after CGS 8216 administration, whereas tremors, increased muscle tone, piloerection and panting were not seen.

None of the cats studied displayed convulsions or died during abstinence.

DISCUSSION

Our results demonstrate that the β-carboline derivative ZK 93426, a benzodiazepine recognition site antagonist (13), fails to precipitate an abstinence syndrome in diazepam-dependent cats when given at a dose that prevents the acute effects of diazepam. In agreement with a recent report (25), we also found that FG 7142, a β-carboline with partial inverse agonist properties (4), does not precipitate a withdrawal syndrome in cats chronically treated with diazepam. In contrast, the BZD receptor antagonist Ro 15-1788 (12), as well as the partial inverse agonists Ro 15-4513 (2, 5, 9, 18, 22, 27) and CGS 8216 (13,26), induce withdrawal signs in diazepam-dependent cats.

These findings suggest that BZD recognition site ligands must fulfill at least two requisites to be able to elicit diazepam abstinence signs: 1) a pharmacological profile corresponding to an antagonist or an inverse agonist and 2) a chemical structure of a BZD or a pyrazoloquinoline derivative. Therefore, the lack of withdrawal signs after a challenge with ZK 93426 or FG 7142 may be related to the β-carboline structure of these drugs. This hypothesis is in line with the view that β-carboline derivatives bind to a domain of the BZD recognition site which is not identical to that recognized by BZD derivatives. Accordingly, several reports indicate that BZD recognition sites are heterogenous and that one of its subsets has specificity for β-carbolines (3, 11, 15). In addition, lesion studies have shown a different cellular distribution of the sites recognized by BZDs and β-carbolines (6,21). Hence, the above data, together with our present results, are consistent with the view that β-carbolines may bind to a site on the GABA receptor complex which is not completely equivalent to the BZD recognition site.

The possibility that pharmacokinetic factors may account for the failure of β-carboline derivatives to precipitate withdrawal signs should be also taken into consideration. Thus, following long-term treatment with diazepam, the brain content of this drug and of its active metabolites may be too high to allow for its rapid displacement from BZD recognition sites by FG 7142 or ZK 93426. However, this possibility appears unlikely since (a) both FG 7142 and ZK 93426 reach sufficiently high cerebral concentrations to antagonize the acute effects of diazepam, at the doses that fail to precipitate withdrawal signs; (b) FG 7142 does not induce abstinence signs in diazepam-dependent cats even at the dose of 20 mg/kg; (c) ZK 93426 is unable to elicit an abstinence syndrome 48 hr after the last dose of diazepam, when the brain levels of this drug and of its active metabolites are significantly reduced; (d) behavioral studies have shown that ZK 93426 and Ro 15-1788 are equipotent in antagonizing different BZD-induced effects (13); and (e) in vitro experiments indicate that ZK 93426 displaces ^{3}H-flunitrazepam binding more potently than Ro 15-1788 and diazepam (13).

In agreement with previous reports in dogs and baboons (17,20) we also observed that CGS 8216 is able to precipitate withdrawal signs in diazepam-dependent cats. However, the abstinence signs were less severe with CGS 8216 than with Ro 15-4513 and Ro 15-1788. This difference may be due to the pharmacokinetic factors that account for the slower onset of action of CGS 8216 (17), since it is well known that a rapid displacement of diazepam from its recognition sites produces more severe withdrawal signs (8,17).

In conclusion, the present study demonstrates that the ability of BZD recognition site ligands to precipitate withdrawal signs in diazepam-dependent cats depends on the chemical structure of the challenge drug, since β-carboline derivatives lack this property.

Moreover, our results provide the first experimental evidence that the acute blockade of BZD recognition sites with the selective antagonist ZK 93426 during chronic diazepam administration does not precipitate a withdrawal syndrome.

Although the molecular mechanisms involved in the lack of withdrawal response after β-carboline challenge are not yet completely understood, this particular property might be used as a new therapeutic approach to reverse BZD overdosage in patients under long-term treatment with BZDs with lower risk of inducing withdrawal signs.

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