

# Acute and chronic effects of the benzodiazepine receptor ligand FG 7142: proconvulsant properties and kindling

Hilary J. Little, David J. Nutt\* & Stuart C. Taylor

Department of Pharmacology, University of Oxford, South Parks Road, Oxford and Department of Psychiatry\*, University of Oxford, Littlemore Hospital, Oxford

- 1 The effects of the acute and chronic administration of the  $\beta$ -carboline benzodiazepine receptor ligand, FG 7142 were studied in mice.
- 2 On acute administration FG 7142 (at doses between 10 and 40 mg kg<sup>-1</sup>) lowered seizure thresholds to infused pentylenetetrazol (PTZ) but showed an unusual dose-response curve in that higher doses had less effect. The duration of action was considerably longer than that of other  $\beta$ -carbolines, such as ethyl- $\beta$ -carboline-3-carboxylate ( $\beta$ CCE).
- 3 During repeated administration, doses of FG 7142 which were initially proconvulsant subsequently produced generalized seizures on average in 60% of animals after 12 once daily treatments. This seemed to be a form of chemical kindling.
- 4 The effects of different drug administration regimes were studied. Once daily dosage was shown to be the optimum for kindling production, and was therefore used for subsequent experiments.
- 5 Kindling lasted for at least one month after 12 single once daily doses of 40 mg kg<sup>-1</sup> (FG 7142).
- 6 The administration of the benzodiazepine antagonist Ro 15-1788 concurrent with FG 7142 prevented kindling. When Ro 15-1788 was given to kindled animals along with a challenge dose of FG 7142, it prevented the expression of kindled seizures. These data show that kindling is mediated via the benzodiazepine receptor.

## Introduction

It is well recognized that tolerance develops to many of the actions of the benzodiazepines. The acute sedative effects rapidly diminish with repeated dosage in both man (Church & Johnson, 1979; Aranko *et al.*, 1983) and animals (Stein & Berger, 1971; File, 1981; Rosenberg & Chiu, 1981). The anticonvulsant effects similarly decline with repeated treatment but over a longer time period (File, 1983). Following the discovery of benzodiazepine receptors, two groups of compounds have been found which compete for the binding sites and prevent the pharmacological effects of the benzodiazepines *in vivo*. One group which includes ethyl -  $\beta$  - carboline - 3 - carboxylate ( $\beta$ -CCE) (Braestrup *et al.*, 1980), FG 7142 (N - methyl -  $\beta$  - carboline - 3 - carboxamide) (Petersen *et al.*, 1982), and DMCM (methyl 6,7,dimethoxy - 4 - ethyl -  $\beta$  - carboline - 3 - carboxylate) (Braestrup *et al.*, 1982), have intrinsic activity which is opposite to that of the benzodiazepines. They lower seizure thresholds (Cowen *et al.*, 1981) or cause convulsions

(Braestrup *et al.*, 1982) and have anxiogenic activity (File *et al.*, 1982; Dorow *et al.*, 1983). Both full and partial agonists of this group of compounds have been described (Braestrup *et al.*, 1982; Petersen *et al.*, 1983). The second group includes the imidazobenzodiazepine Ro 15-1788 (ethyl - 8 - fluoro - 5,6 - dihydro - 5 - methyl - 6 - oxo - 4H - imidazo - [1,5-a] [1,4] benzodiazepine - 3 - carboxylate; Hunkeler *et al.*, 1981). At low doses these do not normally possess anticonvulsant activity against convulsants that do not bind to the benzodiazepine receptor, yet they potently antagonize benzodiazepine actions (Hunkeler *et al.*, 1981; Nutt *et al.*, 1982a). These also antagonize the actions of the first group i.e. the  $\beta$ -carbolines (Nutt *et al.*, 1982a). In order to distinguish these two types the first group have come to be known as 'inverse agonists' (Polc *et al.*, 1982) or 'contragonists' (Nutt, 1983) and the second group as conventional or 'neutral' antagonists. One of the contragonists, FG 7142, which is proconvulsant

(Petersen *et al.*, 1983) and anxiogenic in animals (Petersen *et al.*, 1982; File & Pellow, 1984) has been shown in man to produce severe anxiety and panic (Dorow *et al.*, 1983).

We have now studied the effects of both acute and chronic administration of this compound in mice and find that, in contrast to benzodiazepines, on repeated administration sensitization to its proconvulsant effects was observed, resulting in the production of convulsions.

## Methods

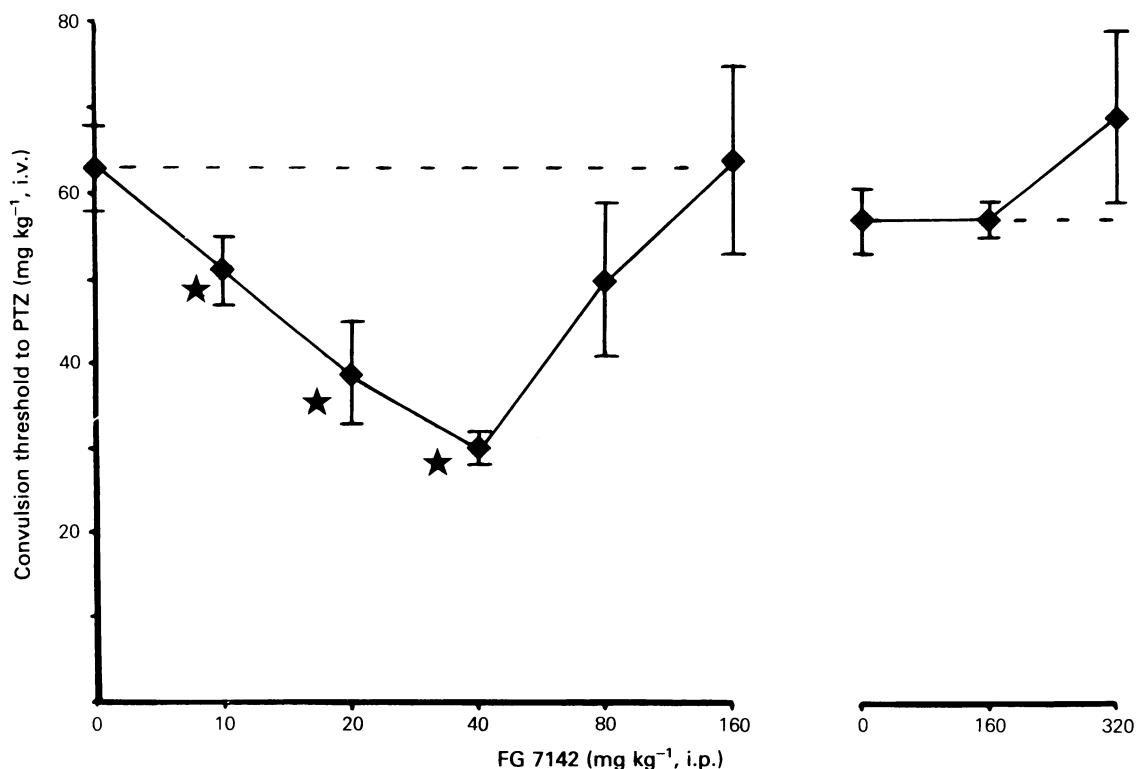
Male CD1 mice (Charles River), weight 32–38 g, were used for both acute and chronic experiments. They were housed in groups of 8 or 16 under controlled lighting and temperature (dark period 20 h 00 min to 08 h 00 min) and given free access to food and water.

FG 7142 (Ferrosan) was suspended in distilled water with one drop of Tween 80 per 10 ml. In all

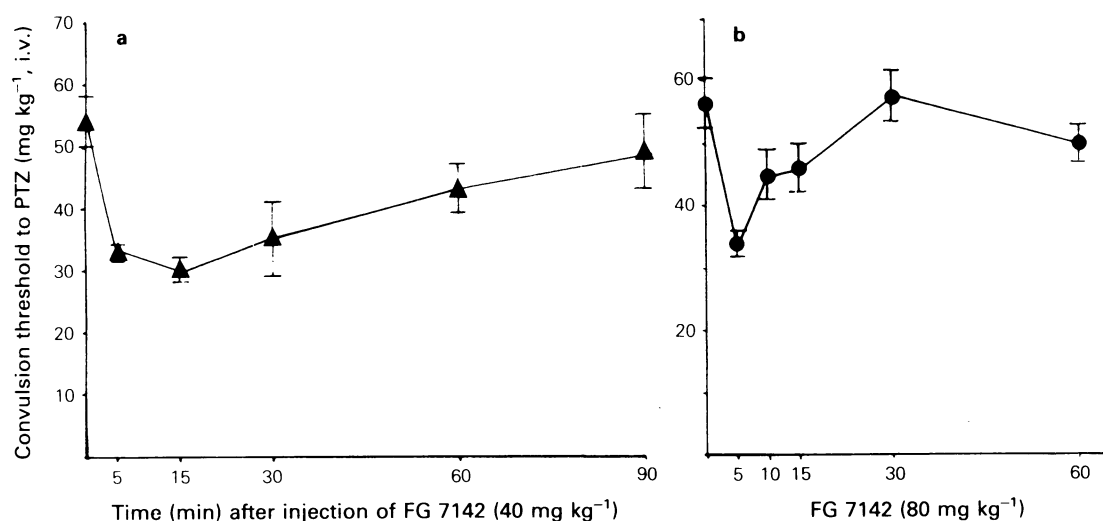
experiments it was given in a volume of 10 ml kg<sup>-1</sup> i.p. Ro 15-1788 (Roche) was suspended in Tween at a concentration of 1 mg ml<sup>-1</sup> and was given i.p.

In the acute experiments seizure threshold testing was performed using an intravenous infusion method (Nutt *et al.*, 1980). Pentylentetrazol (PTZ) (Sigma) 10 mg ml<sup>-1</sup> in normal saline was infused at a rate of 1.1 ml min<sup>-1</sup>. The latency to the onset of myoclonic jerking was timed and seizure thresholds in mg(PTZ) kg<sup>-1</sup> (mouse) were calculated. Myoclonic jerking was defined as repeated contractions, initially of the fore limbs and neck muscles, later extending to the hind limbs.

In the chronic treatment experiments, FG 7142 was given on 3 different schedules, three times a day (10 h 00 min, 16 h 00 min, 22 h 00 min), once daily (20 h 00 min – 22 h 00 min) and three times a week (Monday, Wednesday and Friday 20 h 00 min – 22 h 00 min). Control mice received Tween vehicle. Mice were watched for 1 h after each injection in open top cages (2' × 1'). Observations were made of the behaviour during this time and the incidence of



**Figure 1** Dose-response curve of the proconvulsant effect of FG 7142 as measured by the lowering of pentylentetrazol (PTZ) seizure threshold. FG 7142 was given i.p. in a vol of 1 ml per 100 g 15 min before an infusion of PTZ (10 mg kg<sup>-1</sup>, 1.1 ml min<sup>-1</sup>) into a tail vein. From the time to onset of seizure activity and the weight of the mouse the PTZ seizure threshold was calculated. Points represent mean seizure threshold with s.d. shown by vertical lines. \*Significantly different from Tween vehicle,  $P < 0.05$ .  $n = 5-6$ .



**Figure 2** Time-response curve for the effect of (a) 40 and (b) 80 mg kg<sup>-1</sup> FG 7142 on pentylenetetrazol (PTZ) seizure thresholds. FG 7142 was given as in legend to Figure 1 and PTZ was infused at the times shown. Values are means with s.e. shown by vertical lines. \*Significantly different from time 0:  $P < 0.05$ .  $n = 5$  in (a) and 6 or 7 in (b).

myoclonic jerks, full generalized seizures and other behaviours (e.g. squeaking) recorded. In addition the presence or absence of postictal hypomotility (postictal depression) was noted. A full generalized seizure was defined as clonic or tonic contractions of all limbs plus loss of righting reflex (i.e. the mice fell onto their side or back).

All injections and observations were made by the same observer and all injections were performed in the same room.

As the once daily treatment seemed optimal for the production of sensitization to the convulsant effects of FG 7142 this schedule was used for subsequent experiments. Generally, these challenge tests were done on day 19, after 6 drug-free days, since at this time the mice were still as sensitive as on day 12

(see later) and there was little likelihood of residual FG 7142. Different doses of FG 7142 (20 and 80 mg kg<sup>-1</sup>) were given to determine dose effects on sensitization, and in some experiments Ro 15-1788 (10 mg kg<sup>-1</sup>) was given with the FG 7142, and, owing to its short half life (Lister *et al.*, 1984) a further dose was given 30 min later.

The significance tests used were the Mann-Whitney 'U' test and Fisher's exact test.

## Results

### Acute experiments

In the acute experiments FG 7142 lowered the seizure threshold to PTZ (Figure 1). This effect was

**Table 1** The interaction between Ro 15-1788 and FG 7142 on pentylenetetrazol (PTZ) seizure thresholds

Drug treatment (mg kg <sup>-1</sup> )	PTZ seizure threshold (mg kg <sup>-1</sup> )	P value
Tween + Tween	57 ± 4 (6)	< 0.001
Tween + Ro (10)	55 ± 3 (6)	
FG (40) + Tween	43 ± 3 (7)	
FG (40) + Ro (10)	62 ± 3 (7)	< 0.05
FG (160) + Tween	57 ± 2 (7)	
FG (160) + Ro (10)	79 ± 9 (7)	

NS

Mice were treated i.p. with Tween vehicle or FG 7142 in the doses shown; 15 min later they were given either Ro 15-1788 or Vehicle/Tween, and PTZ seizure thresholds were measured 15 min later. Numbers are mean ± s.e. mean with number of animals in parentheses.

maximal at a dose of  $40 \text{ mg kg}^{-1}$  and higher doses showed less proconvulsant activity. The 160 and  $320 \text{ mg kg}^{-1}$  dose did not significantly change the convulsion threshold ( $P > 0.05$ ). Since  $40 \text{ mg kg}^{-1}$  produced the maximal seizure threshold lowering effect this dose was used for chronic treatments.

The time course of the proconvulsant effect of  $40 \text{ mg kg}^{-1}$  FG 7142 is shown in Figure 2. Proconvulsant activity was maximal between 5 and 30 min and was lost by 90 min. A dose of  $80 \text{ mg kg}^{-1}$  showed a rapid onset of proconvulsant effect which quickly disappeared, so that control values were regained by 30 min (Figure 2).

Pretreatment of the mice with Ro 15-1788 completely prevented the proconvulsant effect of FG 7142 and revealed an increase in threshold at  $160 \text{ mg kg}^{-1}$  (Table 1).

### Chronic treatment

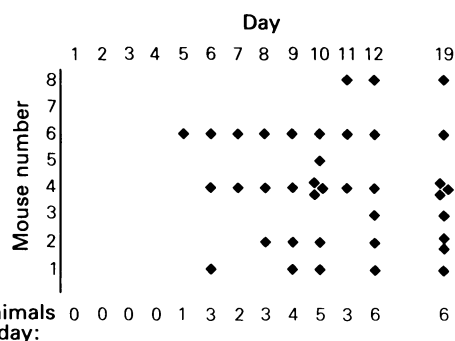
Chronic treatments caused an alteration in the effects of FG 7142. Using 3 times a day treatments no behavioural effects were obvious after the first dose, but by day 3 all animals were showing brief myoclonic jerks of the head and neck. On day 4 generalized seizure activity was noted (Table 2) accompanied by squeaking. Seizure duration was 10–20 s. In general, seizures were observed once after each injection, with a latency which tended to be consistent within mice but which varied between mice ( $16 \pm 1.7 \text{ min}$ , mean  $\pm$  s.e.mean). In one mouse on days 5 and 6 two seizures were observed. The number of mice showing seizures increased to 6/8 by day 6 (see Table 2). It can also be seen that mice were more sensitive to the first injection (10 h 00 min) each day. After day 6, injections were stopped for six days and then the mice were given a further dose on day 12, when they still showed increased sensitivity to the convulsant effects of the drug.

With once daily treatment seizure activity was not noted until day 5 (Figure 3). The number of mice

**Table 2** The effect of repeated 3 times daily administration of FG 7142 ( $40 \text{ mg kg}^{-1}$ )

Injection time	Day No.						
	1	2	3	4	5	6	12
10 h 00 min	0	0	0	2	3	6	—
16 h 00 min	0	0	0	1	2	5	5
22 h 00 min	0	0	0	0	3	3	—

The number of mice out of a total of 8 showing generalized seizures after each injection is shown. No treatment was given between days 7–11 inclusive.



**Figure 3** This indicates the generalized seizures experienced by each individual mouse following once daily injections, of FG 7142 ( $40 \text{ mg kg}^{-1}$ ). The bottom row of numbers is the daily total of mice convulsing. No injections were given on days 13–18 inclusive.

then convulsing increased, until by day 12, 6/8 had shown generalized seizures. The latency to convulsion was less than after three injections/day ( $10 \pm 1.3 \text{ min}$ , mean  $\pm$  s.e.mean), ( $P < 0.01$ , compared with three injections/day), but again did not shorten with repeated administration. When the mice were retested after a week without injections they appeared to be even more sensitive than on day 12. The duration of seizure activity increased to between 30–40 s and a period of obvious postictal depression was observed. Two mice had multiple generalized seizures (Figure 3) and one (No. 6) exhibited a prolonged generalized tonic clonic convulsion that resulted in death. A separate group was given 12 days' treatment and when challenged 30 days after the last dose, equally marked sensitization was observed (Table 3).

With three times a week treatment the first seizure activity was observed after the 4th injection. After the 12th injection 4/8 convulsed.

These results reveal that, far from tolerance developing to the proconvulsant effect of FG 7142, a progressive enhancement of effect was observed with each treatment schedule. Since once daily administration seemed to be optimal and most economical for the production of sensitization, this was used for further studies. A cumulative chart of the total number of animals convulsing on this schedule in all experiments is given in Figure 4.

In a separate study, Ro 15-1788 ( $10 \text{ mg kg}^{-1}$ ) was given concurrently with each FG 7142 injection, and, a further dose was given 30 min later (see Methods). No convulsions were observed during the 12 days of drug treatment (Table 3). Furthermore, when these mice were tested with FG 7142 ( $40 \text{ mg kg}^{-1}$ ) alone after a 7 day drug-free period no evidence of sensitization was observed. When mice that had been treated with FG 7142 over 12 days (such that 5/8

**Table 3** The effects of various treatments on sensitization to seizures produced by FG 7142

<i>Treatment</i>	<i>Challenge</i>	<i>Result (No. of animals showing full convulsions)</i>
FG 7142 40 mg kg <sup>-1</sup> 12 days	FG 7142 40 mg kg <sup>-1</sup>	7/8
FG 7142 40 mg kg <sup>-1</sup> days + Ro 15-1788 10 mg kg <sup>-1</sup> × 2 12 days	FG 7142 40 mg kg <sup>-1</sup> Day 19	0/8
FG 7142 40 mg kg <sup>-1</sup> 12 days	FG 7142 40 mg kg <sup>-1</sup> + Ro 15-1788 10 mg kg <sup>-1</sup> × 2	0/8
FG 7142 40 mg kg <sup>-1</sup> 1 dose	FG 7142 40 mg kg <sup>-1</sup> Day 12	1/8
FG 7142 40 mg kg <sup>-1</sup> 12 days	Tween	0/8
Tween	Day 13	0/8
12 days	FG 7142 40 mg kg <sup>-1</sup> Day 13	0/8
FG 7142 40 mg kg <sup>-1</sup> 12 days	FG 7142 40 mg kg <sup>-1</sup> Day 42	5/8
FG 7142 20 mg kg <sup>-1</sup> 11 days	FG 7142 20 mg kg <sup>-1</sup> Day 12	0/8
FG 7142 20 mg kg <sup>-1</sup> 12 days	FG 7142 20 mg kg <sup>-1</sup> Day 19	0/8
FG 7142 80 mg kg <sup>-1</sup> 11 days	FG 7142 80 mg kg <sup>-1</sup> Day 12	3/8
FG 7142 80 mg kg <sup>-1</sup> 12 days	FG 7142 80 mg kg <sup>-1</sup> Day 19	5/7

Injections were all made by the intraperitoneal route. The treatment doses of FG 7142 were given once a day for the number of days shown, as were the Tween injections. Ro 15-1788 was given in two doses of 10 mg kg<sup>-1</sup>, 30 min apart, the first dose being given at the same time as the FG 7142.

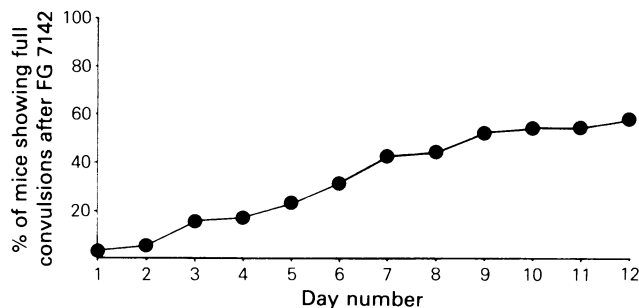
convulsed) were tested on day 19 with FG 7142 (40 mg kg<sup>-1</sup>) given with Ro 15-1788 (10 mg kg<sup>-1</sup>) as before, no seizures were observed.

When FG 7142 was given on day 1 and the mice were retested on day 12, only 1/8 convulsed (Table 3).

Repeated injections of Tween did not sensitize mice to the convulsant effects of FG 7142. A single FG 7142 injection a day after 12 daily Tween treat-

ments produced no overt seizure activity (Table 3). Following kindling with FG 7142 for 12 days a Tween injection on day 13 produced no convulsions (Table 3).

Finally, the effects of repeated treatments with doses of 20 and 80 mg kg<sup>-1</sup> FG 7142 were examined using once daily treatment. The 20 mg kg<sup>-1</sup> dose produced no seizure activity during the 12 days' treatment or when the mice were rechallenged on day



**Figure 4** Cumulative total of mice convulsing after each dose of FG 7142 (40 mg kg<sup>-1</sup>) i.p. given once daily; *n* = 48.

19 (Table 3). Seizures were observed in the 80 mg kg<sup>-1</sup> group such that 3/8 convulsed on day 12 (Table 3) and one died after having had a seizure. On day 19 these animals were rechallenged with 80 mg kg<sup>-1</sup> and 5/7 convulsed ( $P < 0.05$  compared with day 12). These mice showed more severe seizure activity than on day 12, since most had more prolonged seizures and some had repeated convulsions (Table 3).

## Discussion

FG 7142 is a proconvulsant  $\beta$ -carboline that has been shown to produce anxiety in man (Dorow *et al.*, 1983). It is useful for behavioural studies because it has a half-life that is considerably longer than that of the earlier  $\beta$ -carbolines such as  $\beta$ -CCE (Cowen *et al.*, 1981) and is effective given by the intraperitoneal route.

The present study showed firstly that FG 7142 possessed an unusual proconvulsant profile on acute administration, and secondly that chronic administration produced sensitization, which can be called chemical kindling by analogy with electrical kindling (Post, 1981).

On acute administration, FG 7142 lowered seizure threshold to an intravenous infusion of PTZ. This confirms the findings of Petersen *et al.* (1983) who demonstrated that FG 7142 lowered the ED<sub>50</sub> to i.p. PTZ. Similar findings have been observed using the PTZ infusion in rats (Nutt & Green, unpublished observations). Such a proconvulsant effect is consistent with the findings that a number of other  $\beta$ -carbolines are either proconvulsant or convulsant (Cowen *et al.*, 1982; Braestrup *et al.*, 1982; Petersen *et al.*, 1983). The antagonism of this effect by Ro 15-1788 showed that the proconvulsant effect is mediated via an interaction at the benzodiazepine receptor.

The acute dose-response curve of FG 7142 (Figure 1) is unusual. The experiments with Ro 15-1788 revealed an anticonvulsant effect of a high dose (Table 1). This may explain why on acute administration FG 7142 is only proconvulsant. In our hands only one mouse of this strain out of several hundred has convulsed on the first injection. Similar observations have been made by others (Petersen, personal communication; Nicholass, personal communication). A reduction in pharmacological effect at high dose has also been reported for  $\beta$ -CCM (Jones & Oakley, 1981) but not DMCM (Braestrup *et al.*, 1982). Interestingly, FG 7142 differs from many of the other  $\beta$ -carbolines in that it has, at high doses, anticonvulsant effects against electroshock seizures (Petersen *et al.*, 1983). Since Ro 15-1788 revealed rather than prevented the anticonvulsant effect seen

at high doses this presumably is not a benzodiazepine receptor-mediated phenomenon. However, it is possible that FG 7142 was potentiating an anticonvulsant effect of Ro 15-1788, which when using the PTZ infusion method is normally only seen at high doses of Ro 15-1788 (Nutt *et al.*, 1982a). The unusual shape of the dose-response curve for the proconvulsant effects of FG 7142 appears to be due to a phenomenon peculiar to the convulsion threshold as we have also measured the effects of FG 7142 on body temperature and with this parameter, the highest doses of the compound did cause greater changes than the lower doses (Taylor *et al.*, 1984).

The time course of the proconvulsant effect of 80 mg kg<sup>-1</sup> shows a short-lived initial proconvulsant action of similar magnitude to that produced for much longer by the 40 mg kg<sup>-1</sup> dose. It is also possible that the rapid return of the seizure threshold to initial values may be due to the activation of compensatory mechanisms in the CNS rather than the direct intrinsic anticonvulsant effect of FG 7142 at higher doses.

The chronic studies showed that under three different treatment regimes, FG 7142 produced an increased effect with repeated dosage. This phenomenon of sensitization to the convulsant and other effects of drugs has been reported, for example, with cocaine (Post & Rose, 1976), amphetamines (Segal & Mandell, 1974) and local anaesthetics such as lignocaine (Post, 1982). Many convulsants which act at the GABA receptor complex also produce kindling (Nutt *et al.*, 1982b). With all these previous studies kindling was shown to produce a quantitative change in sensitivity to the drug. The findings with FG 7142 are the first in which a qualitative change in behaviour is seen. On acute dosage, FG 7142 is only proconvulsant, whereas on chronic administration it becomes a full convulsant.

There are a number of possible explanations of this type of kindling. The efficacy of FG 7142 may have altered so that it changed from a partial to a full contragonist. Tolerance may have developed to the anticonvulsant properties of FG 7142 seen at high doses; but if this were the case one would have expected more rapid kindling to 80 mg kg<sup>-1</sup> FG 7142, which was not seen. A further explanation is that benzodiazepine receptor binding was altered in the kindled mice. These options are now being investigated.

The possibility that pharmacokinetic factors may underlie these observations has to be considered. Such a phenomenon may explain kindling to cocaine (Ho *et al.*, 1977). Several of the above findings argue against this. Drug accumulation is unlikely in that FG 7142 has a short half-life in mice, with full recovery from the proconvulsant effects being seen within 90 min. Furthermore, the three times a day schedule

of chronic administration did not produce faster kindling than once daily administration. Additionally, with acute treatment the higher doses had less proconvulsant activity (Figure 1) so that if more drug was entering the brain, reduced rather than enhanced effects might be expected. Also, the persistence of and, in the daily injection schedule, apparent enhancement of the state of sensitization four weeks after the last injection makes drug accumulation a very unlikely explanation.

If, as seems likely, these findings have a pharmacodynamic basis they further emphasize the consistently opposite actions of benzodiazepine agonists and 'contragonists'. These have previously been demonstrated by *in vitro* binding (Braestrup & Nielsen, 1981; Mohler, 1982), *in vitro* electrophysiological (Nutt *et al.*, 1982a; Polc *et al.*, 1982; Little, 1984) and acute behavioural studies (Cowen *et al.*, 1981; File *et al.*, 1982; Nutt *et al.*, 1982b). We have now shown that chronic contragonist treatment also produces opposite changes to those seen during chronic benzodiazepine treatment since kindling may be considered the reverse of tolerance.

The phenomenon of kindling may have considerable importance in our understanding of clinical conditions such as anxiety and epilepsy. If endogenous ligands for the benzodiazepine receptor exist they may be either benzodiazepine-like (anxiogenic, anticonvulsant) or contragonist-like (anxiogenic, proconvulsant). If they are the latter type, and are released episodically, perhaps in response to pronounced stress, their effects might be amplified in the same manner as those of FG 7142. Such a phenomenon could explain the production and persistence of anxiety states and some forms of epilepsy. In this context it is especially interesting that recently Ro 15-1788 (10 mg kg<sup>-1</sup>) has been shown to retard the development of electrically kindled seizures (Robertson & Riives, 1983).

We thank Ferrosan for the gift of FG 7142, and Roche for Ro 15-1788. This work was supported by the Wellcome Trust, and the E.P. Abraham Fund. It has previously been presented in abstract form to the British Pharmacological Society.

## References

- ARANKO, K., MATILLA, M.J. & SEPPALA, T. (1983). Development of tolerance and cross-tolerance to the psychomotor actions of lorazepam and diazepam in man. *Br. J. clin. Pharmacol.*, **15**, 545-552.
- BRAESTRUP, C. & NIELSEN, M. (1981). GABA reduces binding of 3H-methyl beta-carboline carboxylate to brain benzodiazepine receptors. *Nature*, **294**, 472-474.
- BRAESTRUP, C., NIELSEN, M. & OLSEN, C.E. (1980). Urinary and brain beta - carboline - 3 - carboxylates as potent inhibitors of brain benzodiazepine receptors. *Proc. natn. Acad. Sci. U.S.A.*, **77**, 2288-2292.
- BRAESTRUP, C., SCHMIECHEN, R., NEEF, G., NIELSEN, M. & PETERSEN, E.N. (1982). Interaction of convulsive ligands with benzodiazepine receptors. *Science*, **216**, 1241-1243.
- CHURCH, M.W. & JOHNSON, L.C. (1979). Mood and performance of poor sleepers during repeated use of flurazepam. *Psychopharmacology*, **61**, 309-316.
- COWEN, P.J., GREEN, A.R., NUTT, D.J. & MARTIN, I.L. (1981). Ethyl beta - carboline - carboxylate lowers seizure threshold and antagonises flurazepam induced sedation in rats. *Nature*, **290**, 54-55.
- DOROW, R., HOROWSKI, R., PASCHELKE, G., AMIN, M. & BRAESTRUP, C. (1983). Severe anxiety induced by FG 7142, a beta-carboline ligand for benzodiazepine receptors. *Lancet*, **ii**, 98-99.
- FILE, S.E. (1981). Rapid development of tolerance to the sedative effects of lorazepam and triazolam in rats. *Psychopharmacology*, **77**, 284-288.
- FILE, S.E. (1983). Tolerance to the anti pentylenetetrazol effects of diazepam in the mouse. *Psychopharmacology*, **79**, 284-286.
- FILE, S.E., LISTER, R.G. & NUTT, D.J. (1982). The anxiogenic action of benzodiazepine antagonists. *Neuropharmacology*, **21**, 1033-1037.
- FILE, S.E. & PELLOW, S. (1984). FG 7142, a  $\beta$ -carboline, has an anxiogenic action in the social interaction test. *Br. J. Pharmacol.*, **82**, 240.
- HO, B.T., TAYLOR, D.C., ESTEREZ, U.S., ENGLERT, L.F. & MCKENNA, M.L. (1977). Behavioural effects of cocaine: Metabolic and neurochemical approach. In *Cocaine and other stimulants*. ed. Ellinwood, E.H. & Kilbey, M.M. pp. 229-240. New York: Plenum.
- HUNKELER, W., MOHLER, H., PIERI, L., POLC, P., BONETTI, E.P., CUMIN, R., SCHAFFNER, R. & HAEFELY, W. (1981). Selective antagonists of benzodiazepines. *Nature*, **290**, 515-516.
- JONES, B.J. & OAKLEY, N.R. (1981). The convulsant properties of methyl beta - carboline - 3 - carboxylate in the mouse. *Br. J. Pharmacol.*, **74**, 884-885.
- LISTER, R.G., GREENBLATT, D.J., ABERNETHEY, D.R. & FILE, S.E. (1984). Pharmacokinetic studies on Ro 15-1788, a benzodiazepine receptor ligand, in the brain of the rat. *Brain Res.*, **290**, 183-186.
- LITTLE, H.J. (1984). The effects of benzodiazepine agonists, contragonists and Ro 15-1788 on the responses of the superior cervical ganglion to GABA *in vitro*. *Br. J. Pharmacol.*, **83**, 57-68.
- MOHLER, H. (1984). Benzodiazepine receptors: differential interaction of benzodiazepine agonists and antagonists after photoaffinity labelling with flunitrazepam. *Eur. J. Pharmacol.*, **80**, 435-436.
- NUTT, D.J., COWEN, P.J. & GREEN, A.R. (1980). On the measurement in rats of the convulsant effect of drugs

- and the changes which follow electroconvulsive shock. *Neuropharmacology*, **19**, 1017–1023.
- NUTT, D.J., COWEN, P.J. & LITTLE, H. (1982a). Unusual interactions of benzodiazepine receptor antagonists. *Nature*, **295**, 436–438.
- NUTT, D.J., COWEN, P.J., BATTS, C.C., GRAHAME-SMITH, D.G. & GREEN, A.R. (1982b). Repeated administration of GABA-antagonist drugs at subconvulsant doses. I. Effects on seizure threshold (kindling). *Psychopharmacology*, **76**, 84–87.
- NUTT, D.J. (1983). Pharmacological and behavioural studies on benzodiazepine antagonists and contragonists. In: *Benzodiazepine recognition site ligands: Biochemistry and Pharmacology*. ed. Biggio, G. & Costa, E. pp. 153–173. New York: Raven Press.
- PETERSEN, E.N., PASCHELKE, G., KEHR, W., NIELSEN, M. & BRAESTRUP, C. (1982). Does the reversal of the anticonflict effect of phenobarbital by beta-CCE and FG 7142 indicate benzodiazepine-receptor mediate anxiogenic properties? *Eur. J. Pharmacol.*, **82**, 217–221.
- PETERSEN, E.N., JENSEN, L.H., HONORE, T. & BRAESTRUP, C. (1983). Differential pharmacological effects of benzodiazepine receptor inverse agonists. In *Benzodiazepine recognition site ligands: Biochemistry and Pharmacology*. ed. Biggio, G. & Costa, E. pp. 57–64. New York: Raven Press.
- POLC, P., BONETTI, E.P., SCHAFFNER, R. & HAEFELY, W. (1982). A three-state model of the benzodiazepine-receptor explains the interactions between the benzodiazepine antagonist Ro 15-1788, benzodiazepine tranquilizers, beta-carbolines, and phenobarbitone. *Arch. Pharmacol.*, **32**, 260–264.
- POST, R.M. & ROSE, H. (1976). Increasing effects of repetitive cocaine administration in the rat. *Nature*, **260**, 731–732.
- POST, R.M. (1981). Lidocaine-kindled limbic seizures: Behavioural implications. In: *Kindling 2*, ed. Wada, J.A. pp. 149–160. New York: Raven Press.
- ROBERTSON, H.A. & RIVES, M.L. (1983). A benzodiazepine antagonist is an anticonvulsant in an animal model for limbic epilepsy. *Brain Res.*, **270**, 380–382.
- ROSENBERG, H.C. & CHIU, T.H. (1981). Regional specificity of benzodiazepine receptor down-regulation during chronic treatment of rats with flurazepam. *Neurosci. Letts.*, **24**, 49–52.
- SEGAL, D.S. & MANDELL, A.J. (1974). Long-term administration of alpha-amphetamine: progressive augmentation of motor activity and stereotypy. *Pharmacology, Biochemistry and Behaviour*, **2**, 249–255.
- STEIN, L. & BERGER, B.D. (1971). Psychopharmacology of 7-chlor-5-(O-chlorophenyl)-1,3-dihydroxy-2H-1,4-benzodiazepine-2-one (Lorazepam) in squirrel monkey and rat. *Arzneim. Forsch.*, **21**, 1073–1078.
- TAYLOR, S.C., LITTLE, H.J., NUTT, D.J. & SELLARS, N. (1984). A benzodiazepine agonist and contragonist have hypothermic effects in rodents. *Neuropharmacology*, (in press).

(Received June 1, 1984.

Revised August 3, 1984.)