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The pharmacological properties of the imidazobenzodiazepine, FG 8205, a novel partial agonist at the benzodiazepine receptor

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1 The pharmacological properties of the benzodiazepine receptor ligand, FG 8205 (7-chloro-5,6-dihydro-5-methyl-6-oxo-3-(5-isopropyl-1,2,4-oxadiazol-3-yl)-4H-imidazol[1,5a][1,4]benzodiazepine) have been examined.

2 FG 8205 potently displaced [^{3}H]-flumazenil binding in rat cortical membranes with a K_i of 3.3 nM, but was inactive at 13 neurotransmitter recognition sites.

3 Consistent with a partial agonist profile, the affinity of FG 8205 for the benzodiazepine recognition site was increased in the presence of γ-aminobutyric acid (GABA, 300 μM) by a degree ($-\log [IC_{50}$ in the presence of GABA/IC_{50} alone] = 0.34) significantly less than found for diazepam (0.46). FG 8205 also potentiated the inhibitory potency of the $GABA_A$-receptor agonist, isoguvacine, on the hippocampal CA1 population spike and, again, the maximum shift ($-\log$ dose-ratio = 0.2) was significantly less than that seen with diazepam (0.4).

4 In anticonvulsant studies, the ED_{50} doses of FG 8205 and diazepam needed to antagonize seizures induced by pentylenetetrazol (PTZ) or by sound in audiogenic seizure prone mice were similar with values of 0.2–0.3 mg kg^{-1}, i.p. However, even high doses of FG 8205 (50 mg kg^{-1}) did not protect against seizures induced by electroshock.

5 FG 8205 released responding suppressed by footshock in a rat operant conditioned emotional response task over the dose range 0.5–50 mg kg^{-1} (i.p.). Similar doses of FG 8205 had a marked taming effect in cynomolgus monkeys. However, measures of sedation and ataxia (as measured by rotarod in the mouse, climbing behaviour in the rat, and by scoring arousal and co-ordination in primates) were slight and only transiently affected by FG 8205, and FG 8205 significantly antagonized the rotarod performance deficit induced by diazepam in the mouse.

6 While the potentiation by FG 8205 of the response to isoguvacine in the rat hippocampal slice and the anxiolytic-like effects of the compound in both rats and primates were reversed by the benzodiazepine receptor antagonist, flumazenil, high doses of the antagonist were able only marginally to block the protective effects of FG 8205 against seizures induced by PTZ in the mouse.

7 Thus, FG 8205 does not show the marked motor impairment characteristic of full agonists at the benzodiazepine receptor, consistent with its partial agonist profile in *in vitro* assay systems. Nevertheless, the compound has sufficient intrinsic activity to maintain high efficacy in anticonvulsant and anxiolytic tests.

Introduction

Recognition sites for benzodiazepines are known to be linked to the γ-aminobutyric $acid_A$ ($GABA_A$)-receptor associated with a chloride channel within the same macromolecular complex (Olsen, 1982; Ehlert *et al.*, 1982; Wong, 1989). Occupation of the site by an agonist, such as diazepam, enhances $GABA_A$-mediated responses by increasing the amount of Cl^- current gated by the binding of a GABA receptor agonist (Study & Barker, 1981).

Benzodiazepines and other compounds capable of interacting with the benzodiazepine recognition site possess a wide range of pharmacological actions: the prototypical full agonist, diazepam, is anxiolytic, sedative, muscle relaxant and anticonvulsant over a narrow dose range (Braestrup *et al.*, 1984; Schneider *et al.*, 1989). A second group of compounds produce effects that are diametrically opposed to those of full agonists; that is they reduce the ability of GABA to increase chloride ion permeability and cause a rightward shift in concentration-effect curves of $GABA_A$-receptor agonists: these are known as inverse agonists (Polc *et al.*, 1982; Braestrup *et al.*, 1982; Haefely *et al.*, 1985; Kemp *et al.*, 1987). Behaviourally, they are convulsant, anxiogenic and heighten arousal. A third class, the benzodiazepine receptor antagonists, such as flumazenil and ZK 93426, are themselves essentially neutral and block the actions of both agonists and inverse agonists (Hunkeler *et al.*, 1981; Bernard *et al.*, 1981; Haefely *et al.*, 1985; Schneider *et al.*, 1989).

The continuum from full agonist to full inverse agonist clearly covers a wide range of possible functional effects *in vivo*: compounds that are partial agonists should be capable of inducing the effects of full agonists which are mediated at low levels of receptor occupancy, but incapable of mimicking the effects of full agonists which require high levels of receptor occupancy (Haefely & Polc, 1986).

There is some evidence that the anticonvulsant effects of the benzodiazepines occur at low benzodiazepine receptor occupation, whilst the myorelaxant and sedative effects require almost 100% occupancy (Jensen & Petersen, 1983). In the present study, evidence is presented indicating that the imidazobenzodiazepine, FG 8205 (7-chloro-5,6-dihydro-5-methyl-6-oxo-3(5-isopropyl-1,2,4-oxadiazol-3-yl)-4H-imidazo-[1,5a][1,4]benzodiazepine) is a partial agonist at the benzodiazepine receptor. In rodents and primates the compound does not induce the marked motor impairment characteristic of full agonists, but nevertheless has high efficacy in tests of anticonvulsant and anxiolytic activity.

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Methods

Animals

Male Sprague-Dawley rats (250–300 g), Swiss Webster (18–20 g) and DBA/2 mice (8–9 g, 21–23 days of age) were obtained from Bantin and Kingman (Hull, U.K.) and caged in groups of 5. Male cynomolgus monkeys (Macaca fascicularis, circa 6 kg, Shamrock Farms, U.K.) were individually housed. All animals were kept under standard laboratory conditions of temperature and humidity and a 12 h light-dark cycle (lights on at 07 h 00 min) with food and water freely available.

Radioligand binding studies

[^{3}H]-flumazenil binding, in vitro Affinity for the benzodiazepine recognition site was determined essentially as described by Wong & Iversen (1985) in membranes derived from rat cerebral cortex. Animals were killed by stunning and decapitation and the whole cortex removed and homogenised in 9 vol of ice-cold 0.32 M sucrose by 10 strokes in a glass teflon homogeniser at 500 r.p.m. All further procedures were carried out at 4°C.

Homogenates were centrifuged at 1000 *g* for 10 min and the supernatant was recentrifuged at 10,000 *g* for 20 min. The P_2 pellet was resuspended in 30 volumes of ice-cold distilled water and centrifuged at 8000 *g* for 20 min. The upper 'buffy coat' and the loosely sedimented pellet were suspended in 20 volumes of ice-cold water and centrifuged at 50,000 *g* for 20 min. The water washing step was repeated 3 times and the resultant pellet stored at −20°C for at least 18 h. On the day of the experiment, the membranes were thawed at room temperature for 30 min, mixed with 20 volumes of 5 mM Tris HCl (pH 7.4) and left at room temperature for a further 30 min, then centrifuged at 50,000 *g* for 10 min. The pellet was resuspended in 20 volumes of the Tris buffer and incubated at room temperature for 15 min before centrifugation. This washing step was repeated three times, prior to resuspension for the binding assay in Krebs buffer (pH 7.4) of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 5, HEPES 20, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.

Benzodiazepine receptors were labelled with 1.0 nM [^{3}H]-flumazenil (100 μl of 10 nM), 100 μl of displacer (test compound) or 30 μM clonazepam (for defining non-specific binding), 50 μl of buffer or 6 mM GABA (for GABA-shift measurements), and 750 μl of membrane homogenate. To control for possible uptake of GABA, 0.1 mM nipecotic acid (final concentration) was added to the assay buffer in all experiments. Duplicate samples containing 0.2 mg protein were incubated for 60 min at 30°C. The binding reaction was terminated by filtration through Whatman GF/B glass fibre filters in a Brandel M24 cell harvester followed by 2 × 5 ml washes with ice-cold 0.9% NaCl. The filters were soaked in 10 ml of Hydrofluor (National Diagnostics) overnight before liquid scintillation counting. Potencies for displacement (IC_{50} or K_i) in the absence or presence of GABA were determined by including at least 5 concentrations of the test compound, and the data analysed by a computer curve fitting programme.

Affinity of FG 8205 for other neurotransmitter recognition sites The affinity of FG 8205 for neurotransmitter recognition sites other than the benzodiazepine receptor was examined in a similar manner to that described for [^{3}H]-flumazenil binding. Details of the radioligands used and the methods for determining non-specific binding are shown in Table 1.

Electrophysiological studies

The ability of benzodiazepine receptor agonists, antagonists and inverse agonists to modulate the inhibitory potency of the $GABA_A$-receptor agonist, isoguvacine, on the CA1 population spike recorded from slices of rat hippocampus was determined.

Male Sprague-Dawley rats (approximately 100 g) were killed by decapitation and their brains rapidly removed. Slices 350 μm thick, from the dorso-medial part of the hippocampus were cut in artificial CSF (aCSF), at room temperature (20°C), with an Oxford vibratome. Slices were placed on a nylon mesh and completely submerged in a small superfusion chamber (volume = 0.3 ml), and continuously superfused with oxygenated aCSF at a rate of approximately 1.5 ml min^{-1}, at room temperature. The aCSF had the following composition (mM): NaCl 124, KCl 5, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 25 and glucose 11.

The Schaffer collateral-commissural pathway was stimulated every 30 s with a metal bipolar electrode, made from two tungsten microelectrodes (TM25-5, Clark electromedical), placed in the stratum radiatum. Population spikes were recorded from the cell body layer of the CA1 pyramidal cells by use of glass micropipettes filled with 3 M NaCl and having resistances of 2–10 MΩ.

In most experiments two slices were placed in the perfusion chamber and their responses recorded in parallel. The recorded potentials were digitised by a Gould OS4040 digital oscilloscope. A BBC microcomputer based system was used to average and measure the peak height of the population spikes. The average of four submaximal control responses was taken and then the perfusing medium changed to one containing isoguvacine by means of a three-way tap. Each concentration of isoguvacine was perfused for a 5 min period, to allow for equilibration within the slice, and the last four responses at each concentration averaged. Increasing concentrations were added cumulatively and the concentration-response curve generated by plotting isoguvacine concentration against % reduction of the population spike. The benzodiazepine receptor ligands under study were perfused for 30 min before and during re-determination of the isoguvacine concentration-response curve. The dose-ratio between the control and drug-treated curves was always measured at the 50% inhibition level. Only one concentration of one ligand was tested on each slice.

Table 1 Details of ligand binding assays

Recognition site	*Radioligand*	*Tissue*	*Non-specific binding determined by:*	*Non-specific conc.* (μmol l^{-1})	*Radioligand conc.* (nmol l^{-1})	*Incub. time* (min)	*Temp.* (°C)
5-HT_{1A}	[^{3}H]-8-OH-DPAT	Cortex	5-HT	10	1	5	37
5-HT_2	[^{3}H]-ketanserin	Cortex	Methysergide	1	2	15	37
5-HT_3	[^{3}H]-quat-ICS 205-930	Cortex	MDL72222	10	0.5	15	4
α_1	[^{3}H]-prazosin	Whole brain	Phentolamine	10	1	30	23
β	[^{125}I]-cyanopindolol	Cortex	(−)-Isoprenaline	200	0.15	20	37
D_2	[^{3}H]-ADTN	Striatum	Dopamine	10	16	20	23
CCK_A	[^{125}I]-BH-CCK	Pancreas	CCK	1	0.05	120	23
CCK_B	[^{125}I]-BH-CCK	Cortex	CCK	1	0.05	120	23
NMDA	[^{3}H]-MK-801	Cortex	TCP	10	2	45	23
Glycine	[^{3}H]-glycine	Cortex/hippo	Glycine	1000	50	30	4
Sigma	[^{3}H]-DTG	G P whole brain	Haloperidol	1	5	90	23
Muscarinic	[^{3}H]-oxotremorine	Cortex	Atropine	100	3	40	30
	[^{3}H]-N-methylscopolamine	Cortex	Atropine	100	0.1	40	30

Anticonvulsant activity

Seizures induced by pentylenetetrazol Mice were injected subcutaneously with pentylenetetrazol (PTZ, 120 mg kg^{-1}, s.c.) and observed for the following 30 min. FG 8205 and diazepam were administered i.p., 30 min before injection of PTZ. Animals not exhibiting tonic seizures during the following 30 min observation period were considered protected. The dose of antagonist giving 50% protection (ED_{50}) was calculated by probit analysis.

Seizures induced by electroshock Tonic seizures were induced in mice by application of electroshock (0.5 mA, 0.2 s) via corneal electrodes. FG 8205 and diazepam were given i.p., 30 min before application of shock and the dose giving 50% protection calculated by probit analysis.

Audiogenic seizures Seizures were induced in 21–23 day-old male DBA/2 mice by exposure for 30 s to a 120 dB, 1.4 kHz bell. FG 8205 and diazepam were given i.p., 30 min before exposure to the sound. Animals not exhibiting a tonic seizure within the 30 s of sound exposure were considered protected. The dose of compound giving 50% protection against each convulsant was calculated by probit analysis.

Anxiolytic studies

Rat conditioned emotional response Rats (250–300 g) were trained to lever-press on a variable interval, VI 60 s schedule for food reinforcement in a standard conditioning chamber (Gerbrand's Instruments) over weekly (five days per week) training sessions. All animals then received daily 20 min conditioning sessions, each session being partitioned into alternating 5 min light (L) and 2.5 min dark (D) periods in a fixed LDLDL sequence. During both types of period (L,D), lever-presses delivered food pellets on a V1 60 s schedule; in the dark periods (D), lever-presses also elicited mild footshock (0.8 mA, 0.5 s) on an independent shock presentation schedule of V1 20 s.

Lever-pressing was suppressed during the dark period and reflects the formation of a conditioned emotional response (CER) as the animals learn to discriminate between light and dark. Rats were tested on extinction days (shock off) separated by 1 or 2 days of baseline re-training (shock on, identical to training sessions). Subjects were injected with carboxymethylcellulose vehicle (0.5% w/v in distilled water) before every training session to eliminate any cues deriving from the injection. Unless stated otherwise, compounds were administered i.p. 40 min before testing. All drug testing was carried out in separate groups of rats (minimum of 8 per group) for each drug or dose tested. Test treatments were given according to a randomised sequence to minimize order effects. Results are expressed as response rates in the light and dark periods.

Taming effect in primates Procedures similar to those first described by Heise & Boff (1961) and Randall *et al.* (1961) were used to detect the taming effect of benzodiazepines in aggressive monkeys. Six individually-housed, adult male cynomolgus monkeys were used; attack or avoidance behaviour directed towards the observer (following (i) sudden approach and (ii) hand clapping nearby), a broom handle and a live garter snake were each rated on a scale of 0–5 to give a maximum total aggression score of 20. Animals were scored by an observer blind to the drug treatment immediately before the i.p. administration of diazepam or FG 8205 and at 30 min intervals thereafter for a 5 h observation period. Animals were subjected to these and other drug treatments usually at one week intervals.

For calculation of the ED_{50} for taming, scores were summed over the period of peak effect and plotted against drug dose on a logarithmic scale. The point at which the dose-response curve was intersected by a line representing a 50% reduction in aggression score was taken as the ED_{50}. A 50% reduction in aggression was defined as a score mid-way between the maximum observed following vehicle treatment and the minimum observed after any drug treatment.

Duration of action was estimated by examination of the time course of taming activity of the dose closest to the calculated ED_{50} value. Duration was defined as the time taken for scores to increase from 50 to 75% of control values.

Sedation and motor co-ordination

Mouse rotarod Groups of male Swiss Webster mice were trained on a rotarod apparatus (diameter of rod = 3 cm) until they were able to remain on the rotating rod (15 rev min^{-1}) for at least 120 s, at which point they moved continuously with the rod, maintaining their position on the top. Animals who fell several times were discarded. Time spent on the rotating rod was then determined at 15, 30, 45 and 60 min after the i.p. injection of FG 8205 or, for comparison, diazepam. The duration of each trial was 2 min.

Rat swimming and climbing performance A platform was positioned 1 m from the start point of a circular swimming pool, 1.32 m in diameter. When plunged into the water at room temperature, rats swam spontaneously and searched for a means of escape. The time taken to locate the platform and then to mount the platform once contact was established, was noted. FG 8205 and diazepam were injected i.p., 30 min before test.

Sedation and ataxia in primates Sedation and ataxia were each scored in male cynomolgus monkeys on a scale of 0–5, according to the ease with which the animals could be aroused and the degree of motor incoordination (for example, when picking up a peanut) and postural instability. The scores were summed to give a combined sedation + ataxia score. Behaviours were scored immediately before i.p. drug administration and every 30 min throughout the subsequent 5 h period by an observer blind to drug and dose conditions.

Drugs

The following compounds were used: FG 8205 (7-chloro-5,6-dihydro-5-methyl-6-oxo-3-(5-isopropyl-1,2,4-oxidiazol-3-yl)-4H-imidazo [1,5-a][1,4]benzodiazepine, Merck Sharp & Dohme Research Laboratories), diazepam (Roche), alprazolam (Upjohn), methyl-6,7-dimethoxy-4-ethyl-β-carboline carboxylate (DMCM, Research Biochemicals Inc.), flunitrazepam (Roche) and flumazenil (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo(1,5a)(1,4)benzodiazepine-3-carboxylate, Roche). Bretazenil (t-butyl(S)-8-bromo-11,12,13,13a-tetrahydro-9-oxo-9H-imadazo[1,5-a]-pyrrolo-[2,1-c][1,4] benzodiazepine-1-carboxylate) and Ro 17-1812(cyclopropylmethyl (5)-8-chloro-12,12a-dihydro-9-oxo-9H,11H-aceto[2,1-C]-imidazo-[1,5-a][1,4]benzodiazepine-1-carboxylate) were synthesized at Merck Sharp & Dohme Research Laboratories.

When administered to rodents, compounds were suspended in 0.5% w/v carboxymethylcellulose in distilled water with the aid of ultrasonification. In the cynomolgus monkey, 5% (w/v) cremophore in 0.9% NaCl was used as the drug vehicle.

Results

Affinities and GABA-shift values for ligands at rat brain benzodiazepine receptors

FG 8205 was about 10 to 20 fold more potent than alprazolam or diazepam and approximately equipotent with flumazenil in displacing [^{3}H]-flumazenil from the benzodiazepine recognition site in rat cortical membranes. Bretazenil was the most potent compound examined with a K_i 86 fold lower than that of diazepam (Table 2).

Table 2 Potencies and γ-aminobutyric acid (GABA)-shift values for ligands at benzodiazepine receptors in rat cerebral cortical membranes

	K_i (nM) *vs* [^3H]-*flumazenil*	*GABA-shift*	*log GABA shift*
FG 8205 (6)	3.3 ± 0.4	2.2 ± 0.10*	0.34
Diazepam (8)	62.0 ± 9.0	2.9 ± 0.06	0.46
Alprazolam (4)	34.0 ± 6.7	2.9 ± 0.18	0.46
Bretazenil (5)	0.7 ± 0.2	1.4 ± 0.12*	0.15
Ro 17-1812 (5)	5.2 ± 0.8	1.7 ± 0.08*	0.23
Flumazenil (5)	5.7 ± 1.1	1.0 ± 0.04*	0
DMCM (3)	3.4 ± 0.5	0.4 ± 0.03*	−0.40

Values are mean ± s.e.mean from (*n*) number of experiments. The GABA-shift is the control IC_{50}/IC_{50} in the presence of 3×10^{-4} M GABA. For direct comparison with the electrophysiological data, the log GABA shift is also given. *Significantly different from value for diazepam, $P < 0.05$ (unpaired *t* test).

The affinity of both diazepam and alprazolam for the benzodiazepine recognition site was increased in the presence of GABA, giving a GABA-shift (−log ratio of IC_{50} values obtained in the presence and absence of GABA) of 0.46. The GABA-shift for FG 8205 (0.34) was significantly less than that of either diazepam or alprazolam ($P < 0.05$, unpaired *t* test), but not as low as that of bretazenil (0.15), flumazenil (0) or DMCM (−0.4) (Table 2). Similar affinity and GABA-shift values were observed for FG 8205 when [^{3}H]-flumazenil binding was carried out in human cortical membranes (results not shown).

Affinity of FG 8205 at other neurotransmitter recognition sites

At concentrations of 10 to 100 μM, FG 8205 did not displace binding by more than 6% at recognition sites for 5-hydroxytryptamine (5-HT) (5-HT_{1A}, 5-HT_2, 5-HT_3), dopamine (D_2), acetylcholine (muscarinic), noradrenaline (α_1 and β-adrenoceptors), glutamate N-methyl-D-aspartate (NMDA), substance P and cholecystokinin (CCK_A and CCK_B), at the σ-recognition site or the strychnine-insensitive glycine modulatory site on the NMDA receptor (results not shown).

Effects of FG 8205 in the rat hippocampal slice

FG 8205 produced a leftward shift of the isoguvacine concentration-response curve (Figure 1) with a threshold concentration of 10 nM. The maximum shift was achieved at 30 nM (−log concentration-ratio = 0.2) and this was maintained up to the highest concentration tested (300 nM). This compares to maximum shifts produced by diazepam and flunitrazepam of 0.41 and 0.38 respectively (Kemp *et al.*, 1987). The partial agonist, bretazenil, also produced a maximum shift (0.2 at 30 nM) which was also less than that of diazepam, whereas the inverse agonist, DMCM, shifted the isoguvacine concentration-response curve to the right, maximally at 1 μM giving a log concentration-ratio of 0.38.

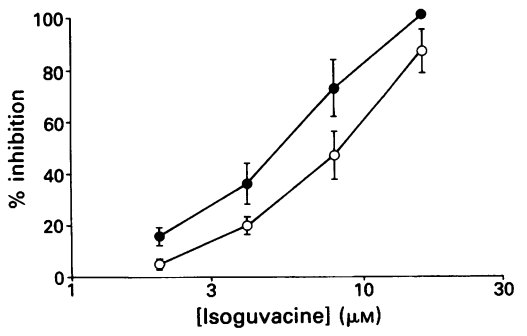

Figure 1 Concentration-response curve for the inhibition of the CA1 population spike in slices of rat hippocampus by isoguvacine before (○) and after (●) 30 min perfusion with FG 8205 (30 nM). Data are shown as the mean percentage inhibition of 4 separate slices; vertical lines indicate s.e.mean.

To confirm that the potentiating effect of FG 8205 was mediated by an action at the benzodiazepine receptor, the effect of the benzodiazepine receptor antagonist, flumazenil was investigated. The increase in the potency of isoguvacine induced by FG 8205 (30 nM) was first determined and then flumazenil (300 nM) added together with FG 8205 for a further 30 min, and the isoguvacine dose-response curve re-determined. The mean −log concentration-ratio produced by FG 8205 was 0.197 ± 0.018 (±s.e.mean, $n = 6$). This was reduced significantly to 0.010 ± 0.017 by flumazenil ($P < 0.001$, paired *t* test).

Anticonvulsant studies

Under the present conditions, PTZ (30–120 mg kg^{-1}) dose-dependently induced tonic seizures in the mouse: the dose calculated by probit analysis to induce seizures in 50% of the animals tested was 94 mg kg^{-1} (Singh *et al.*, 1990). For antagonist studies, a dose of 120 mg kg^{-1} PTZ was chosen since this was the minimum dose that reliably induced seizures in 100% of the control animals.

The ED_{50} doses of diazepam and FG 8205 given 30 min before testing against tonic seizures induced in the mouse by pentylenetetrazol or by sound in audiogenic seizure prone DBA/2 mice were similar (Table 3). Diazepam was about 10 fold weaker against seizures induced by electroshock than against the convulsant action of PTZ or sound. However, FG 8205 did not protect against electroshock even at the high dose of 50 mg kg^{-1}.

When co-administered with diazepam, flumazenil (1–40 mg kg^{-1}) dose-dependently antagonized the protection against PTZ (120 mg kg^{-1}), such that the ED_{50} for diazepam increased from 0.39 ± 0.035 mg kg^{-1} (mean of 4 separate determinations ± s.e.mean) in vehicle-treated controls to 1.8 mg kg^{-1} ($n = 2$) and 4.7 ± 1.25 mg kg^{-1} ($n = 4$, $P < 0.01$, 2-tailed *t* test) in the presence of doses of 20 mg kg^{-1} and 40 mg kg^{-1} flumazenil, respectively. In contrast, 40 mg kg^{-1} flumazenil had little effect on the protective effect of FG 8205 against PTZ, the ED_{50} dose increasing by only 1.3 fold from 0.34 ± 0.087 mg kg^{-1} ($n = 3$) to 0.45 ± 0.173 mg kg^{-1} ($n = 3$, $P > 0.05$, two-tailed *t* test). Flumazenil (1–40 mg kg^{-1}) alone was without significant effect on PTZ-induced seizures (results not shown).

Effects of benzodiazepine ligands in the rat conditioned emotional response test

After approximately six weeks of training, lever pressing was completely suppressed by footshock during the dark phase of the conditioned emotional response (CER) test. Response suppression was subsequently maintained in the absence of footshock when extinction sessions were interspersed on a twice

Table 3 Anticonvulsant potency of FG 8205 and diazepam

	Anticonvulsant ED_{50} (mg kg^{-1}) vs		
	PTZ	*Audiogenic seizures*	*Electroshock*
FG 8205	0.26 (0.18–0.34)	0.18 (0.13–0.24)	>50
Diazepam	0.39 (0.31–0.47)	0.18 (0.02–0.04)	3.0 (1.7–5.2)

Groups of 8 mice were given FG 8205 or diazepam 30 min before injection of pentylenetetrazol (PTZ), exposure to sound or electroshock and the number of animals convulsing noted. ED_{50} values were determined by probit analysis. 95% confidence limits are given in parentheses.

weekly basis with three days of training in the presence of shock.

At a dose of 10 mg kg^{-1}, diazepam significantly increased the response rate in the dark to a level not significantly different from the rate in the light phase. However, the increase in dark phase responding occurred over a narrow dose range. Response rate decreased in both light and dark phases following a dose of 20 mg kg^{-1} diazepam (Figure 2a). A very similar pattern of responding was seen with the benzodiazepine receptor agonist, alprazolam (Figure 2b). In contrast, FG 8205 (0.2–50 mg kg^{-1}) dose-dependently increased the dark phase response rate over a wide dose range and without suppression of responding in the light (Figure 2c). Indeed, FG 8205 tended to increase the light phase response rate, although the effect was not statistically significant. The estimated ED_{50} dose of FG 8205 (the dose increasing the dark phase response rate by 50% of the maximum increase seen) when given intraperitoneally was 1.3 mg kg^{-1}. At the ED_{50} dose, the dark phase response rate was highest in animals injected immediately before the start of the test and declined steadily with increasing pretreatment time (Figure 3). However, a significant increase in response rate was still present when the compound was given 180 min before testing. Given orally 40 min before testing, the ED_{50} for FG 8205 was 5 mg kg^{-1} (Figure 4).

The ability of the benzodiazepine receptor antagonist, flumazenil to antagonize the effects of FG 8205 and diazepam in the CER test was also examined. When co-administered with 5 mg kg^{-1} FG 8205 or diazepam, 40 min before test, flumazenil (10 and 20 mg kg^{-1}) dose-dependently antagonized the increase in dark phase response rate induced by both compounds (Figures 5 and 6).

In contrast to the marked release of suppressed responding by FG 8205, the benzodiazepine partial agonist, bretazenil (0.2–50 mg kg^{-1}), induced only a very moderate increase in dark phase response rate at all doses tested (Figure 2d). When co-administered with 5 mg kg^{-1} diazepam, 40 min before test, bretazenil (0.2–5 mg kg^{-1}) antagonized the diazepam-induced increase in dark phase response rate (Figure 7). FG 8205 (0.2–25 mg kg^{-1}) did not alter the increase in dark phase response rate induced by 5 mg kg^{-1} diazepam (results not shown).

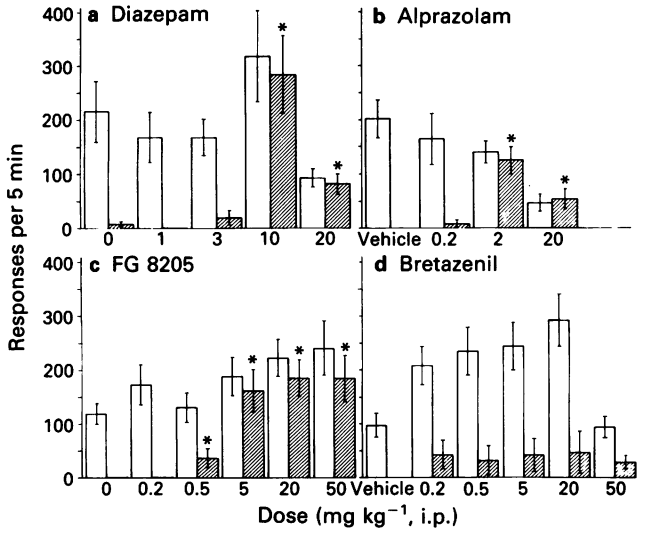

Figure 2 The effects of benzodiazepine receptor agonists in the rat conditioned emotional response test. Columns indicate the mean lever pressing response rate and bars s.e.mean of 8 or more animals per dose group during the light (open columns) and dark (hatched columns) periods of the test. The dark period was associated with punishment (footshock) during training. Drugs were administered i.p. 40 min before testing: (a) diazepam; (b) alprazolam; (c) FG 8205; (d) bretazenil. * Significantly different from vehicle-treated control dark response rate, $P < 0.05$ (ANOVA followed by Dunnett's t test).

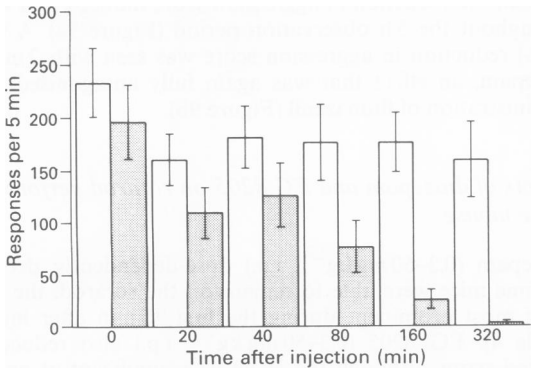

Figure 3 The duration of action of FG 8205 in the rat conditioned emotional response test. Columns indicate the mean lever pressing response rate and bars s.e.mean of 8 or more animals per dose group during the light (open columns) and dark (stippled columns) periods of the test. The dark period was associated with punishment (footshock) during training. FG 8205 (1.3 mg kg^{-1}) was administered i.p. immediately before (0) or at 20, 40, 80, 160, or 320 min before testing.

Taming effect of benzodiazepines in primates

Administration of FG 8205 (0.3–5 mg kg^{-1}, i.p.) induced a marked and dose-dependent reduction in aggression scores by

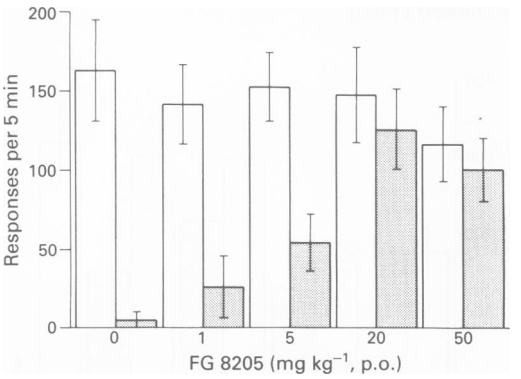

Figure 4 The oral activity of FG 8205 in the rat conditioned emotional response test. Columns indicate the mean lever pressing response rate and bars s.e.mean of 8 or more animals per dose group during the light (open columns) and dark (stippled columns) periods of the test. The dark period was associated with punishment (footshock) during training. FG 8205 was administered orally, 40 min before testing.

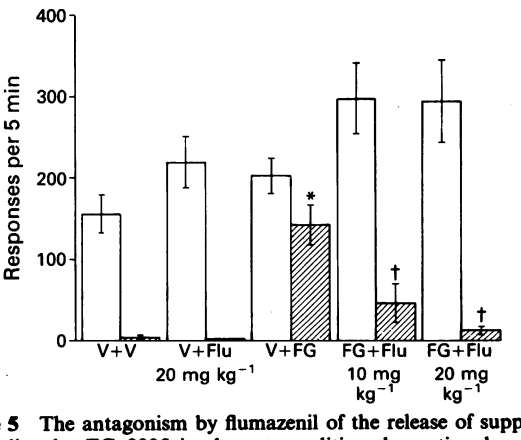

Figure 5 The antagonism by flumazenil of the release of suppressed responding by FG 8205 in the rat conditioned emotional response test. Columns indicate the mean lever pressing response rate and bars s.e.mean of 8 or more animals per group during the light (open columns) and dark (hatched columns) periods of the test. The dark period was associated with punishment (footshock) during training. V = drug vehicle; FG = FG 8205 (5 mg kg^{-1}); Flu = flumazenil, 10 or 20 mg kg^{-1}. Drugs were administered i.p. 40 min before testing. * Significantly different from vehicle-treated control dark phase response rate, $P < 0.05$; † significantly different from dark phase response rate of animals given only FG 8205, $P < 0.05$ (ANOVA followed by Dunnett's t test).

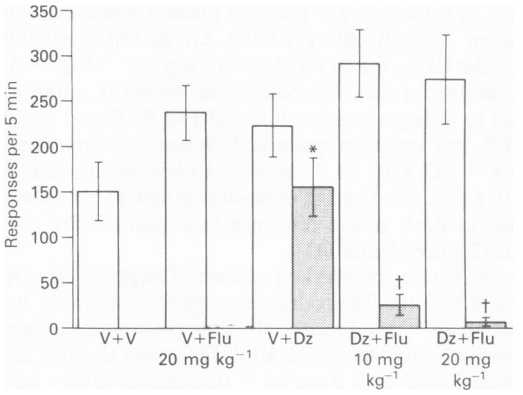

Figure 6 The antagonism by flumazenil of the release of suppressed responding by diazepam in the rat conditioned emotional response test. Columns indicate the mean lever pressing response rate and bars s.e.mean of 8 or more animals per group during the light (open columns) and dark (stippled columns) periods of the test. The dark period was associated with punishment (footshock) during training. V = drug vehicle; Dz = diazepam (5 mg kg^{-1}); Flu = flumazenil, 10 or 20 mg kg^{-1}. Drugs were administered i.p. 40 min before testing. * Significantly different from vehicle-treated control dark phase response rate, $P < 0.05$; † significantly different from dark phase response rate of animals given only diazepam, $P < 0.05$ (ANOVA followed by Dunnett's t test).

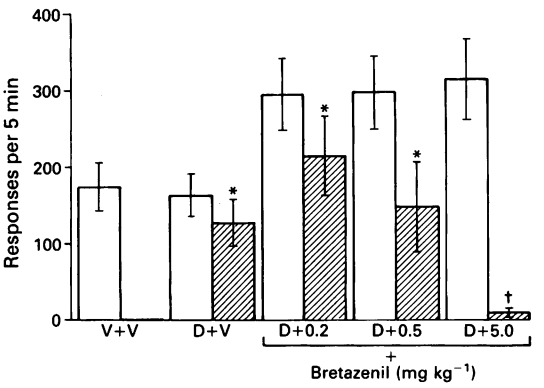

Figure 7 The antagonism by bretazenil of the release of suppressed responding by diazepam in the rat conditioned emotional response test. Columns indicate the mean lever pressing response rate and bars s.e.mean of 8 or more animals per group during the light (open columns) and dark (hatched columns) periods of the test. The dark period was associated with punishment (footshock) during training. V = drug vehicle; D = diazepam (5 mg kg^{-1}). Drugs were administered i.p. 40 min before testing. * Significantly different from vehicle-treated control dark response rate, $P < 0.05$; † significantly different from dark response rate of animals given only diazepam, $P < 0.05$ (ANOVA followed by Dunnett's t test).

comparison to vehicle treatment throughout the 5 h observation period. The taming effect induced by the lowest dose of FG 8205 (0.1 mg kg^{-1}) was the least pronounced, but still significant for up to 2 h after administration (Figure 8a). In contrast, taming was not induced by low doses of diazepam (0.1–0.3 mg kg^{-1}). A dose of 1 mg kg^{-1} diazepam transiently reduced aggression scores between 0.5 and 1.5 h, but only the highest dose (5 mg kg^{-1}) produced marked and sustained taming (Figure 8b).

Estimation of the ED_{50} doses for both FG 8205 and diazepam as the dose reducing the maximum difference between vehicle and drug-treated animals by 50% gave values of 0.1 and 1 mg kg^{-1}, respectively. At the ED_{50} doses, the time taken for the taming score to drop from 50% to 25% of the maximum effect was about 3 h for FG 8205 and 5 h for diazepam.

In a separate experiment, the ability of flumazenil to block the taming effect of FG 8205 and diazepam was examined.

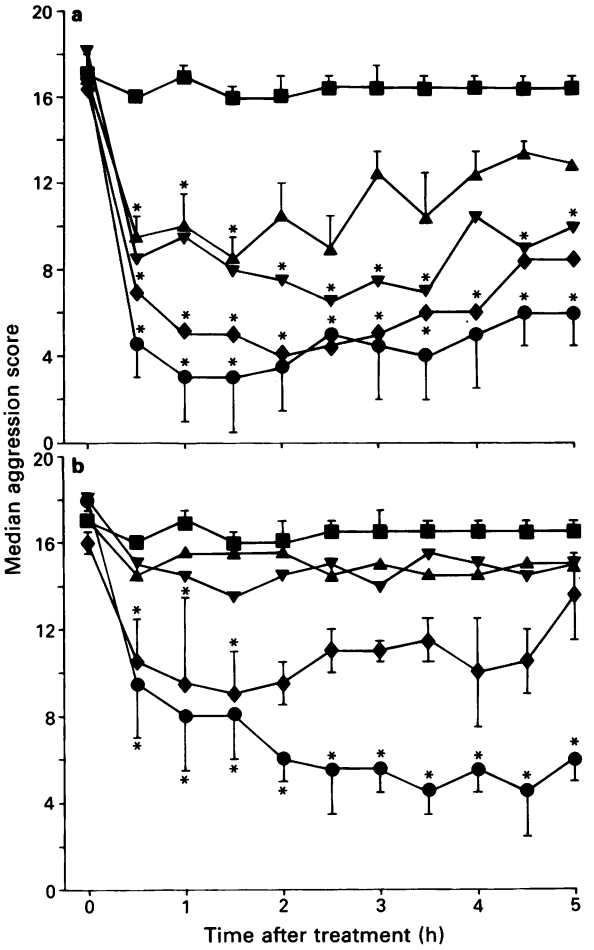

Figure 8 The effect of FG 8205 on aggressive behaviour in cynomolgus monkeys. Attack and avoidance behaviour to the observer (following sudden approach or clapping of hands), a broom handle or a live garter snake was rated on a ranked intensity scale, allowing calculation of the median aggression score of the six animals used. Observations were made immediately before and at 30 min intervals after i.p. injection of (a) FG 8205 or (b) diazepam over a five hour period. (■) Vehicle; (▼) 0.1 mg kg^{-1}; (▲) 0.3 mg kg^{-1}; (◆) 1 mg kg^{-1}; (●) 5 mg kg^{-1} FG 8205 (a) or diazepam (b). Vertical lines indicate the semi-interquartile range. * Significantly different from vehicle-treated control scores, $P < 0.05$ (Mann-Whitney U test).

Treatment with 0.2 mg kg^{-1} FG 8205 reduced aggression scores to about 41% of the pretreatment baseline for up to 3.5 h. Co-administration of flumazenil (5 mg kg^{-1}, i.p.) fully blocked the reduction in aggression score induced by FG 8205 throughout the 5 h observation period (Figure 9a). A similar (53%) reduction in aggression score was seen with 2 mg kg^{-1} diazepam, an effect that was again fully antagonised by co-administration of flumazenil (Figure 9b).

Effects of diazepam and FG 8205 on rotarod performance in the mouse

Diazepam (0.3–50 mg kg^{-1}, i.p.) dose-dependently decreased the time mice were able to remain on the rotarod, the effects being most prominent during the first 15 min after injection (Table 4). FG 8205 (0.3–50 mg kg^{-1}, i.p.) also reduced the rotarod score, although the effect was significant at only one dose level (30 mg kg^{-1}) and one time point (15 min after injection). Indeed, when animals were co-administered FG 8205 (0.2–25 mg kg^{-1}) with diazepam (20 mg kg^{-1}), the impairment in rotarod performance determined 30 min after injection was dose-dependently reduced, although even after 25 mg kg^{-1} FG 8205 complete reversal of the diazepam-induced deficit was not achieved (Figure 10).

Table 4 Effect of FG 8205 and diazepam on rotarod performance in the mouse

Compound	Dose ($mg\,kg^{-1}$)	Time time on rotarod (s) 15	30	45	60 min
Vehicle	—	109 ± 7	115 ± 5	120	120
FG 8205	0.3	92 ± 13	115 ± 5	120	109 ± 8
	1	91 ± 10	116 ± 3	120	120
	3	115 ± 4	116 ± 4	107 ± 9	120
	10	79 ± 12	95 ± 13	100 ± 11	116 ± 4
	30	71 ± 9*	107 ± 7	109 ± 8	113 ± 7
	50	89 ± 12	99 ± 9	108 ± 8	112 ± 8
Vehicle	—	120	112 ± 8	120	120
Diazepam	0.3	120	120 ± 3	107 ± 9	116 ± 4
	1	113 ± 5	120	114 ± 6	120
	3	98 ± 11	105 ± 11	115 ± 5	116 ± 3
	10	64 ± 11*	71 ± 13	94 ± 11	103 ± 10
	30	15 ± 6**	45 ± 16	70 ± 19	74 ± 18
	50	40 ± 14**	33 ± 14**	61 ± 19*	69 ± 20*

Mice were tested for their ability to remain on the rotarod at 15, 30, 45 and 60 min after drug injection. Values are means ± s.e.mean of 8–10 animals per group.
* Significantly different from vehicle-treated controls, $P < 0.05$ (Kruskal Wallis ANOVA followed by Mann-Whitney U test).

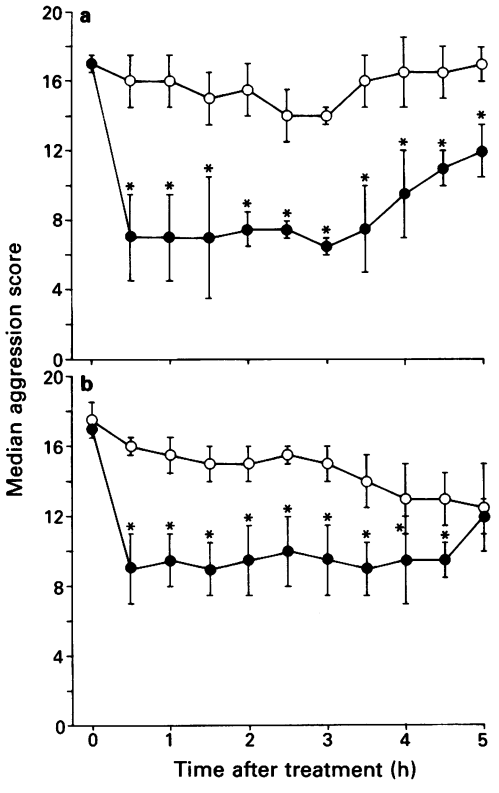

Figure 9 The antagonism by flumazenil of the anti-aggressive effect of (a) FG 8205 and (b) diazepam in cynomolgus monkeys. Flumazenil ($5\,mg\,kg^{-1}$) was co-administered i.p. with either (a) FG 8205 ($0.2\,mg\,kg^{-1}$) or (b) diazepam ($2\,mg\,kg^{-1}$) and aggressive behaviour rated as described in the legend to Figure 8. (○) FG 8205 or diazepam; (●) FG 8205 or diazepam + flumazenil. * Significantly different from animals given only FG 8205 or diazepam (Mann-Whitney U test).

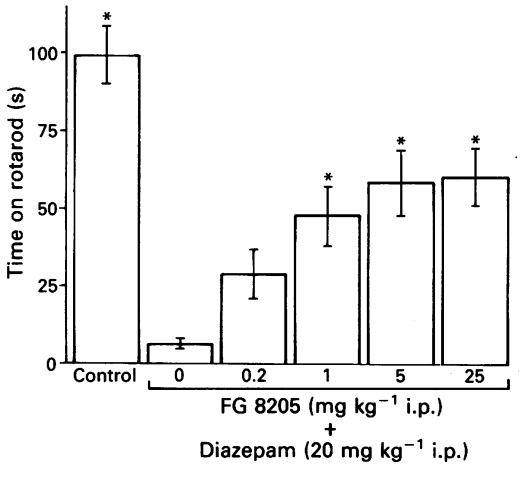

Figure 10 Antagonism by FG 8205 of the rotarod deficit induced in the mouse by diazepam. Animals were trained to remain on a rotarod revolving at 15 r.p.m. for 120 s and then co-administered various doses of FG 8205 with diazepam ($20\,mg\,kg^{-1}$) i.p. Thirty minutes later, they were returned to the rotarod and the latency to fall noted. Columns represent the mean time spent on the rotarod and bars s.e.mean of at least 8 animals per group. * Significantly different from animals given only diazepam, $P < 0.05$ (Kruskal Wallis ANOVA followed by Mann-Whitney U test).

Effects of diazepam and FG 8205 on swimming and climbing performance in the rat

Rats in their home cage were calm and inactive following the acute administration of diazepam ($1–50\,mg\,kg^{-1}$) or FG 8205 ($1–50\,mg\,kg^{-1}$). However, neither FG 8205 ($1–50\,mg\,kg^{-1}$) nor diazepam ($1–50\,mg\,kg^{-1}$) significantly altered the time taken to locate the platform of a swim maze (Table 5). On the other hand, diazepam markedly increased the time taken to mount the platform: animals given a dose of $30\,mg\,kg^{-1}$ took more than 20 s to escape the water. In contrast, FG 8205 increased the time taken to mount the platform by a maximum of only 2.4 s (Table 5).

Sedation and ataxia in primates

Scores for sedation and ataxia in cynomolgus monkeys were obtained at each 30 min observation period following drug administration. Cumulative scores are shown in Table 6. A slight, but significant increase in the score was seen only after a dose of FG 8205 ($5\,mg\,kg^{-1}$), 50 fold higher than the minimum effective dose to induce taming. In contrast, a high sedation/ataxia score was seen at the lowest dose of diazepam that produced a marked and sustained taming effect.

Discussion

The imidazobenzodiazepine, FG 8205 potently inhibited [^{3}H]-flumazenil binding in rat cortical membranes, but was without significant affinity for thirteen neurotransmitter

Table 5 Effects of diazepam and FG 8205 on swimming performance in the rat

	Dose ($mg\,kg^{-1}$)	Latency to reach platform (s)	Latency to mount platform (s)
Vehicle	—	33.9 ± 6.2	1.8 ± 0.3
Diazepam	1	32.8 ± 7.8	2.9 ± 0.3
	3	42.7 ± 6.9	2.9 ± 0.3
	10	47.1 ± 6.8	6.8 ± 1.9*
	30	47.6 ± 8.3	21.4 ± 2.7*
	50	49.1 ± 6.2	27.6 ± 1.8*
Vehicle	—	32.7 ± 5.2	1.7 ± 0.2
FG 8205	1	34.5 ± 4.4	2.9 ± 0.3*
	3	37.4 ± 3.6	3.9 ± 0.5*
	10	43.8 ± 6.6	4.0 ± 0.4*
	30	42.7 ± 5.3	4.1 ± 0.6*
	50	35.4 ± 6.7	3.9 ± 0.5*

Rats were plunged into a swimming pool 1 m from a visible platform and the time taken to (a) locate the platform and (b) mount the platform was noted. Rats were tested 30 min after i.p. injection of either diazepam or FG 8205. Values are means ± s.e.mean of 8–10 animals per group. * Significantly different from vehicle-treated controls, $P < 0.05$ (ANOVA followed by Dunnett's t test).

Table 6 Sedation and ataxia induced by FG 8205 or diazepam in primates

Dose ($mg\,kg^{-1}$)	Summed sedation and ataxia score/30–210 min FG 8205	Diazepam
0.1	0	0
0.3	1.0 ± 2	0
1.0	3.5 ± 4.5	2.5 ± 2
5.0	5.5 ± 4*	18.5 ± 6*

Animals were scored for sedation and ataxia on a scale of 0–5 according to the ease with which animals could be aroused and their motor co-ordination. Summed scores were compared with those of vehicle-treated animals by use of Kruskal Wallis analysis of variance of ranks followed by Mann-Whitney U-tests. The summed score of vehicle-treated animals was 0.
* $P < 0.05$ compared with control treatment.

recognition sites, indicating its very high selectivity for the benzodiazepine receptor. As with diazepam and alprazolam, compounds considered to be full benzodiazepine receptor agonists, the affinity of FG 8205 for the [^{3}H]-flumazenil recognition site was increased by the presence of GABA. However, the magnitude of the 'GABA shift' suggests that FG 8205 has a level of efficacy less than that of diazepam, but greater than that of the benzodiazepine partial agonists, bretazenil and Ro 17-1812 (Kemp *et al.*, 1987). The GABA shifts of the latter two compounds were, in turn, greater than that of the neutral antagonist, flumazenil, which had no GABA shift, and opposite to that of the beta-carboline inverse agonist, DMCM.

In the rat hippocampal slice, benzodiazepine receptor agonists dose-dependently potentiate the inhibitory potency of the selective $GABA_A$-receptor agonist, isoguvacine, on the CA1 population spike. Previous studies (Kemp *et al.*, 1987) have shown that this potentiation closely parallels the degree of benzodiazepine receptor occupation and thus, partial agonists are unable to produce the same maximum potentiation as full agonists. Consistent with its low GABA shift, FG 8205 gave a maximum potentiation of the isoguvacine-induced inhibition which was significantly less than that produced by diazepam or alprazolam. The shift in potency induced by FG 8205 was, in this case, similar to that seen with bretazenil and Ro 17-1812, but again opposite to that produced by DMCM.

Whilst the intrinsic activity of FG 8205 is low compared to diazepam and alprazolam, sufficient efficacy remains for the genesis of both anticonvulsant and anxiolytic effects in a number of test situations. Thus, the compound was equipotent with diazepam against seizures induced by PTZ or by sound in audiogenic seizure prone mice, two models that are highly sensitive to benzodiazepines. Diazepam was itself a much weaker antagonist of seizures induced by electroshock and, perhaps consistent with the lower intrinsic activity and inactivity of other partial benzodiazepine receptor agonists (Petersen *et al.*, 1984; Haefely, 1984; Schneider *et al.*, 1989), FG 8205 was inactive in this test even at a dose 200 fold greater than that required to block seizures induced by sound or PTZ.

In the conditioned emotional response task, the full benzodiazepine receptor agonists, diazepam and alprazolam, were able to increase lever pressing in the dark phase of the test associated with punishment (footshock) during training. However, the response to both compounds was biphasic, the higher doses used giving lower response rates in both dark and light, probably reflecting the onset of sedation. FG 8205 dose-dependently increased the dark phase response rate over a wide dose range and without suppression of responding in the light. In contrast to FG 8205, the somewhat lower efficacy benzodiazepine receptor agonist, bretazenil (at least in terms of its lower GABA shift) induced only a very moderate increase in dark phase response rate in the CER test. In addition, bretazenil was able to antagonize the increase in dark phase response rate induced by diazepam, while FG 8205 had no such effect. In view of their seemingly modest differences in *in vitro* measures of efficacy, the differences in the behavioural profiles of FG 8205 and bretazenil are perhaps surprising. They are, nevertheless, not inconsistent with the benzodiazepine 'efficacy' hypothesis.

Compounds that are anxiolytic in man are often able to release suppressed responding in rodent behavioural models similar to the CER test described here: it is likely, therefore, that FG 8205 would also be anxiolytic in man. This is further supported by the taming effects of the compound in aggressive cynomolgus monkeys. Like diazepam, FG 8205 reduced attack and avoidance behaviour to aversive stimuli. Although FG 8205 was approximately 10 fold more potent than diazepam, the duration of action of the compound at its ED_{50} dose was shorter than that of diazepam (3 h cf 5 h for diazepam).

Sedation and ataxia are marked features of the behavioural and neurological response to benzodiazepine receptor agonists in both rodents and primates. In the mouse, this was clearly reflected in the disruption of performance on the rotarod following treatment with diazepam. In contrast to diazepam, FG 8205 induced only a very mild and transient performance deficit at high doses. In the rat, FG 8205 decreased activity in the home cage, but only slightly impaired the ability of animals to climb from a swimming pool, a task that was again markedly disrupted by diazepam. Similarly, in primates sedation and ataxia induced by FG 8205 was slight and transient after doses ten fold higher than the minimum required for a significant taming effect.

It has been hypothesized that benzodiazepine-mediated sedation reflects the occupation of the receptor by high efficacy agonists in regions of low receptor reserve (Jensen & Petersen, 1983; Haefely & Polc, 1986). For example, with *in vivo* radioligand binding techniques, the anticonvulsant effects of diazepam and the benzodiazepine partial agonist, bretazenil have been found to occur at receptor occupancies of 5% and 40%, respectively (Potier *et al.*, 1988). In contrast, diazepam did not induce marked motor deficits until 35% occupancy had been achieved, whilst even at an occupancy of 90–100%, bretazenil was without myorelaxant effects. Hence, the low liability of FG 8205 to induce sedation and ataxia in rodents and primates is entirely predictable from the low intrinsic activity of the compound in *in vitro* biochemical and electrophysiological test systems. The 'partial agonist' or 'efficacy' theory also predicts that compounds of low intrinsic efficacy

will antagonize the actions of full agonists. Consistent with this hypothesis, FG 8205 clearly attenuated the rotarod performance deficit induced in the mouse by diazepam.

However, the varied pharmacological profile of partial benzodiazepine receptor agonists invites alternative explanations: the different behavioural effects of these compounds could equally reflect different degrees of efficacy at more than a single benzodiazepine recognition site. The anticonvulsant profile of FG 8205 suggests the existence of one subtype that is insensitive to the benzodiazepine receptor antagonist, flumazenil. Thus, while the potentiation by FG 8205 of the response to isoguvacine in the rat hippocampal slice and the anxiolytic-like effects of the compound in both rats and primates were reversed by flumazenil, high doses of the antagonist were able to block only marginally the protective effects of FG 8205 against seizures induced by PTZ in the mouse. Preliminary studies also suggest this to be the case in the rat (Tricklebank, unpublished observations). Since the anticonvulsant effects of diazepam were reversed by flumazenil under an identical dosing regime, it seems unlikely that the insensitivity of FG 8205 can be explained by insufficient occupation of the receptor by the antagonist. On the other hand, although cloning experiments have strongly supported the existence of multiple benzodiazepine receptors (Pritchett *et al.*, 1989a,b; Khrestchatisky *et al.*, 1989), none have yet been shown to be flumazenil-insensitive. It remains to be seen whether FG 8205 will play a useful role in providing the functional evidence necessary to substantiate the pharmacological relevance of putative subtypes of the $GABA_A$-receptor.

In conclusion, *in vitro* test systems have shown FG 8205 to have many of the characteristics of a partial agonist at the benzodiazepine receptor. In rodents and primates, the compound lacks the sedation/ataxia profile of full agonists. Intrinsic activity is nevertheless sufficient to endow FG 8205 with good activity in anxiolytic paradigms, although it is not clear whether its anticonvulsant effects reflect partial agonist properties at the benzodiazepine receptor; at a subtype of the benzodiazepine receptor or (despite its lack of activity in many other neurotransmitter binding assays) an interaction at non-benzodiazepine sites involved in the generation or expression of seizures. Other studies have indicated that mice are less likely to become tolerant to the anticonvulsant effects of FG 8205 than they are to diazepam and show little evidence of a dependence syndrome on withdrawal from chronic treatment (Tricklebank *et al.*, unpublished observations). As such, FG 8205 should have a better side-effect profile in man than many of the currently available full benzodiazepine receptor agonists.

We thank Hoffmann-La Roche and Upjohn for the gift of drugs.

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