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The pharmacological properties of Y-23684, a benzodiazepine receptor partial agonist

[1]Hiroshi Yasumatsu, Yasuto Morimoto, Yoshinobu Yamamoto, Shuzo Takehara, Takemi Fukuda, Tohru Nakao & Michihide Setoguchi

Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd., 955 Koiwai, Yoshitomi-cho, Chikujo-gun, Fukuoka 871, Japan

1 The pharmacological properties of a benzodiazepine receptor (BZR) partial agonist, Y-23684 were investigated in comparison with those of diazepam, a conventional BZR full agonist.

2 Y-23684 and diazepam showed high and selective affinity for the BZR with K_i values of 41 and 5.8 nM, respectively.

3 In contrast to diazepam, variability was noted in the anticonvulsive potency of Y-23684 depending on convulsants (bicuculline, pentylenetetrazol and maximal electrical shock). Y-23684 produced the most potent protective effect against bicuculline in rats and mice with ED_{50}s of 1.3 and 1.2 mg kg^{-1}, respectively.

4 In rat conflict models (Geller-Seifter and water-lick tests), Y-23684 produced an antipunishment action at doses 2–4 times lower than diazepam. In contrast to diazepam, Y-23684 did not affect unpunished responding up to 50 mg kg^{-1} in the Geller-Seifter test.

5 In other rat models of anxiety (social interaction and elevated plus-maze tests), Y-23684 was as efficacious as and ten fold more potent than diazepam. In a mouse model of anxiety (exploration (light/dark box) test), Y-23684 was as efficacious and two fold less potent as diazepam. In these paradigms, Y-23684 showed a selective anxiolytic profile over a wide dose-range without loss of efficacy and sedative action.

6 The impairment of motor coordination (rotarod) and potentiation of CNS depressants (ethanol and hexobarbitone) by Y-23684 was much weaker than that of diazepam.

7 These results suggest that Y-23684 would be a potent and selective anxiolytic agent in man with less side-effects than conventional BZ-anxiolytics.

Keywords: Y-23684; benzodiazepine receptor partial agonist; diazepam; Geller-Seifter test; water-lick test; social interaction test; elevated plus-maze test; exploration test

Introduction

Since the discovery of specific binding sites in mammalian brain for benzodiazepines (BZs) (Möhler & Okada, 1977; Squires & Braestrup, 1977), numerous pharmacological analyses have permitted great progress in the field of BZ research. The history of BZs as the standard treatment for anxiety disorders during the past three decades indicates that they have been highly effective agents. However, considerable concern has been expressed regarding their ability to produce a series of undesirable features (i.e. sedation, muscle relaxation, amnesia, interactions with alcohol/barbiturates and dependency-liability).

A huge variety of novel agents interacting with the BZR have been synthesized over the past 10 years in order to understand the molecular mechanisms by which these agents produce their biological responses, and ultimately to develop non-sedative 'anxioselective' agents. These advances enabled two hypotheses to be proposed. One is BZR multiplicity, two receptor subtypes, $BZ(omega)_1$ and $BZ(omega)_2$, exist in different brain areas, subserving different physiological functions. The introduction of BZ_1-selective imidazopyridines, zolpidem and alpidem (Depoortere *et al.*, 1986; Zivkovic *et al.*, 1990), has fuelled the debate about the functional consequences of receptor multiplicity (Doble & Martin, 1992). An alternative approach is the development of BZR partial agonists (Petersen, 1987; Müller, 1988; Haefely *et al.*, 1990). It is now generally accepted that central BZR is an integral part of the $GABA_A$ receptor channel, a hetero-oligometric glycoprotein complex (Jenck *et al.*, 1992). Experimental evidence indicates that BZR ligands can be classified with different efficacy on a continuum from full agonists to inverse agonists, according to their modulatory effects on GABAergic transmission. Partial agonists are of interest because of their lower positive intrinsic efficacy, between those of full agonists represented by diazepam and the zero intrinsic efficacy of pure antagonists such as flumazenil, which might be sufficient to maintain the anxiolytic and anticonvulsant responses, but insufficient to induce unwanted side-effects seen with conventional full agonists. The triazopyridazine, CL 218,872 (Lippa & Crichett, 1979), the imidazobenzodiazepinone, bretazenil (Martin *et al.*, 1988), the β-carboline, abecarnil (Stephens *et al.*, 1990) and other classes of compounds have to date been claimed to be BZR partial agonists, even though some of them also discriminate between BZ_1 and BZ_2 sites. The results in animal studies with these drugs have demonstrated their distinguishable 'anxioselective' behavioural profile from full agonists to confirm the plausibility of the BZR partial agonist theory.

Based on this hypothesis, the functional properties of a series of condensed pyridazinone derivatives have been studied in our laboratories with a view to designing new anxiolytics (Nakao *et al.*, 1990; 1991a,b; 1992). Among them, Y-23684, (±)-2-(4-chlorophenyl)-5,6-dihydro-[1]benzothiepino-[5,4-c]pyridazin-3(2H)-one 7-oxide (Figure 1, molecular wt.: 356.83), showed a BZR partial agonistic property that possessed a lower intrinsic efficacy than diazepam on the BZR/GABA-chloride channel complex in an electrophysiological assay, which was similar to CL 218,872 and bretazenil (Yakushiji *et al.*, 1990). In the present paper, we describe the preclinical pharmacological profile of Y-23684 in comparison with diazepam.

[1] Author for correspondence.

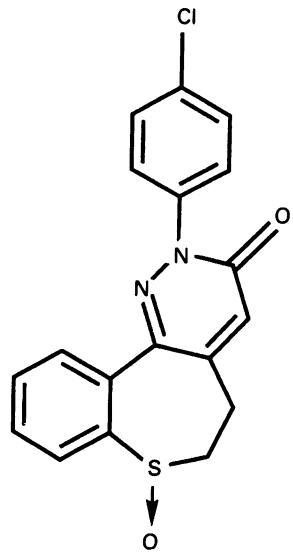

Figure 1 Chemical structure of Y-23684.

Methods

Animals

Wistar rats (170–250 g), Wistar-King A rats (350–450 g), Lister hooded rats (270–350 g), ddY mice (20–24 g) and BKW mice (30–38 g) were obtained from Seiwa Experimental Animals, Ltd. (Fukuoka, Japan). Wistar and Lister hooded rats were housed in groups of 5; ddY and BKW mice were housed in groups of 10–20. They were allowed food and water *ad libitum*. Wistar-King A rats were housed individually, and were food deprived to 85% of their free feeding body weight. Unless stated otherwise, animals were kept in environmentally controlled conditions (06 h 00 min–18 h 00 min lights on; 24 ± 1°C). All behavioural assessments were carried out between 09 h 00 min and 18 h 00 min.

Drugs

Y-23684 and diazepam were synthesized at our medicinal chemistry department. They were suspended in 0.5% methylcellulose (MC) solution, and administered orally (p.o.) in a volume of 1 or 2 ml kg^{-1} for rats and 10 ml kg^{-1} for mice. Unless stated otherwise, Y-23684 and diazepam were administered 3 and 1 h before each test in rats, respectively. In mouse studies, both compounds were administered 1 h before each test. The pretreatment time in both species for each compound was fixed to attain the maximum plasma concentration based upon pharmacokinetic studies (unpublished observations). All control groups were treated with an equal volume of vehicle. (+)-Bicuculline (Sigma) was dissolved in 0.1 N HCl. Pentylenetetrazol (Aldrich) and ethanol (Ishizu Seiyaku) were dissolved in distilled water. Hexobarbitone (Teikoku Kagaku) was suspended in 0.5% MC solution.

BZ binding

A binding study of [^{3}H]-diazepam to crude synaptosomal membranes of rat cerebral cortex was performed, based on the method described previously (Möhler & Okada, 1977). A total volume of 1.0 ml consisted of 900 µl of crude synaptosomal membranes containing approximately 1 mg of protein, 50 µl of [^{3}H]-diazepam (final concentration: 2 nM) and compounds dissolved in 50 µl of ethanol incubated at 0°C for 10 min. Membrane bound radioactivity was determined by filtration through Whatman GF/B filters followed by liquid scintillation counting. Nonspecific binding was determined in the presence of 1 µM unlabelled diazepam.

Radioligand binding to other neurotransmitter receptors

The affinity of the test compounds for other neurotransmitter receptors was assayed with rat brain homogenates. All the studies summarized in Table 1 were performed based on methods described previously; $GABA_A$ receptors (Beaumont *et al.*, 1978), dopamine D_2 receptors (Creese *et al.*, 1977), α_1-adrenoceptors (Greengrass & Bremner, 1979), α_2-adrenoceptors (U'Prichard *et al.*, 1979), 5-HT_{1A} receptors (Hall *et al.*, 1985), 5-HT_2 receptors (Peroutka & Snyder, 1979) and muscarinic cholinoceptors (Yamamura & Snyder, 1974).

Anticonvulsant studies

Male Wistar rats in groups of 7–21 or male ddY mice in groups of 7–14 were used. The animals were challenged with bicuculline (0.6 mg kg^{-1}, i.v.: Lippa & Regan, 1977; Rastogi & Ticku, 1986), pentylenetetrazol (150 mg kg^{-1}, s.c. for mice and 100 mg kg^{-1}, i.p. for rats: Tsumagari *et al.*, 1978) or maximal electrical shock (MES, AC 2000 V, 12.5 mA, 0.2 s for mice) delivered through bilateral corneal electrodes. The criteria used to indicate convulsant responses were tonic extension (hindlimb for mice and forelimb for rats) in bicuculline- and MES-seizures, and lethality in pentylenetetrazol-seizures. The anticonvulsant potency of Y-23684 and diazepam were estimated as ED_{50} values and their 95% confidence limits.

Geller-Seifter conflict test

The procedure was based on the method described previously (Geller & Seifter, 1960). Food-deprived male Wistar-King A rats were trained to press a lever for milk reinforcement in operant chambers. The schedule consisted of alternations of four 10 min fixed ratio (FR) 20 unpunished periods and four 5 min FR1 punished periods signalled by a white noise. During the punished periods, a 300 ms foot-shock was given in association with milk delivery. To study anticonflict effects, the intensity of foot-shock (400–800 µA) was adjusted for each rat to suppress total lever presses during the four punished periods to under 10. Y-23684 and diazepam were administered 2.5 and 0.5 h before the start of tests, respectively. In this test, the pretreatment time for each compound was modified from other rat studies so that the maximum plasma concentration could be obtained in the middle of a 60 min period.

Water-lick conflict test

The procedure was modified from the method described previously (Vogel *et al.*, 1971). Male Wistar rats were deprived of water for 72 h, and were placed in the test chamber and allowed to drink from the water spout. Licking was automatically accompanied by a 100 V, 0.2–0.3 mA, 300 ms electrical stimulus across the grid floor and spout every twenty licks. After the rat received the first electrical stimulus, the number of stimuli was recorded automatically during the subsequent 3 min test. All rats were naive to the test chamber.

Social interaction test

The procedure was essentially as described by File (1980). The test apparatus used for the detection of rat social interaction behaviour and exploratory behaviour consisted of an open-topped perspex box (51 × 51 × 20 cm(H)) with 17 cm square areas marked on the floor of the box. The light intensity of the arena floor was 1200 lux, and that of the experimental sound-proof room at the floor level was

100 lux. Male Lister hooded rats were allowed at least 4 h for adaptation to the new environment. Effects of the test compounds were determined by treating each pair of rats, which were naive to the test arena.

The test was started by placing each pair of rats, from separate housing cages, in opposite corners of the arena, and their behaviours were observed over a 10 min period by video recording, leaving them undisturbed. All behavioural assessments were made from the recordings, with the observer unaware of the original treatment. Social interactions between the rats were determined by timing sniffing of the partner, climbing over or crawling under the partner, genital investigation of the partner and following of the partner. Additionally, exploratory locomotion was measured as the number of line crossings marked on the floor of the test arena.

Elevated plus-maze test

The elevated plus-maze test is based on the hypothesis that exposure to an elevated and open maze alley leads to an approach-avoidance conflict that is considerably stronger than that evoked by exposures to a closed alley (Chopin & Briley, 1987). The procedure is valid in various rat strains, heights of alley or walls of the closed arms, judgement of entries (whole arms or end sections) and lighting conditions (Handley & Mithani, 1984; Pellow *et al.*, 1985; Meert & Janssen, 1989; Costall *et al.*, 1989b; Singh *et al.*, 1991). Since these studies suggested that a change of these factors should affect the intensity of aversive environmental stimuli, we further modified the experimental conditions to demonstrate a higher environmental aversion.

Naive male Lister hooded rats were used. Behavioural testing was conducted between 13 h 00 min and 17 h 00 min in a darkened sound-proof room illuminated with dim red lights (5 lux at the floor level), and animals were allowed at least 4 h for adaptation to the new environment. The apparatus consisted of a plus-shaped maze constructed of perspex, elevated 70 cm from the floor. It comprised two closed arms which had transparent sides and an end 10 cm high and two open arms. Both types of arms (45 × 10(W) cm) were marked into two equal sections, and the furthermost (end) sections of open arms were illuminated brightly (1200 lux) with white bulb lights. Individual rats were placed on the centre square of the apparatus facing an open arm and observed over the 10 min test period by video recording, leaving the rats undisturbed. All behavioural assessments were made from the recordings with the observer unaware of the original treatment, and the time spent on the end sections of open arms was noted.

Exploration (light/dark box) test

The procedure was performed based on the method as described previously (Costall *et al.*, 1989a). Naive BKW mice which had been kept for at least 2 weeks under a reversed 12 h light/dark cycle with lights off at 07 h 00 min were used, and behavioural testing was conducted between 13 h 00 min and 17 h 00 min (dark period) in a darkened sound-proof room illuminated with dim red lights (5 lux at the floor level). Animals were allowed at least 4 h for adaptation to the new environment.

The apparatus used for the detection of changes in exploratory behaviours consisted of an open-topped metal box (45 × 45 × 27 cm(H)) lined into 9 cm squares on the floor, two-fifth painted black and illuminated under a dim red light (less than 5 lux at the centre of the arena) and partitioned from the remainder of the box which was brightly illuminated (1000 lux). An opening 7.5 × 7.5 cm located at the floor level in the centre of the partition allowed access between the two compartments. At the start of testing, individual mice were placed in the centre of the brightly lit area of the test box.

The behaviour of mice was recorded over a 5 min period by video recording, leaving them undisturbed. All behavioural assessments were made from the recordings with the observer unaware of the original treatment, and three behaviours were noted: (i) the time spent in the light section (s), (ii) the number of line crossings in the light and dark sections and (iii) the number of exploratory rearings in both sections.

Motor incoordination: rotarod

Male ddY mice and male Wistar rats in groups of 10 were used. Untrained mice were placed on the rotarod (2.8 cm in diameter, 11 r.p.m.). In rat studies, only rats trained previously to stay on the rotarod (5 cm in diameter, 5 r.p.m.) for more than 3 min were used. In both species, the number of animals which dropped off the rotarod within 1 min was determined to estimate ED_{50} values and their 95% confidence limits.

Interactions with alcohol and barbiturates

Male ddY and male Wistar rats in groups of 7–14 were used. The ability of the test compounds to potentiate the action of a subnarcotic dose of ethanol (30% at 10 ml kg^{-1}, i.p. for mice and 25% at 100 ml kg^{-1}, i.p. for rats) or that of hexobarbitone (40 mg kg^{-1}, i.p. for mice and 60 mg kg^{-1}, i.p. for rats) were examined using the loss of righting reflex as a behavioural index. The number of animals which lost the righting reflex for more than 10 s was determined, and ED_{50} values and their 95% confidence limits were estimated.

Statistics

Mean ± s.e.mean were calculated and presented for each treated group. Statistical differences between two groups were made by Aspin-Welch's t-test (Welch, 1949) in the Geller-Seifter test, single factor ANOVA followed by the LSD test in the water-lick conflict test, single factor ANOVA followed by Dunnett's t-test in the social interaction and exploration test, and Kruskal-Wallis ANOVA followed by the Mann-Whitney U-test (two-tailed) in the elevated plus-maze test. In each case the accepted level of significance was $P<0.05$. The ED_{50} values and their 95% confidence limits were determined by probit analyses.

Results

Radioligand binding to BZ and other neurotransmitter receptors

In membranes prepared from rat cerebral cortex, Y-23684 displaced [^{3}H]-diazepam in a concentration-dependent manner. Drug competition studies demonstrated that Y-23684 displayed a high affinity for the ^{3}H-labelled sites (K_i = 41 nM). Under the same experimental conditions, diazepam exhibited a higher affinity (K_i = 5.8 nM) than Y-23684. However, Y-23684 and diazepam did not displace appreciably the appropriate tracers labelling $GABA_A$ receptors, dopamine D_2 receptors, α_1-adrenoceptors, α_2-adrenoceptors, 5-HT_{1A} receptors, 5-HT_2 receptors nor muscarinic cholinoceptors even at a final concentration of 100 μM (Table 1).

Anticonvulsant potency of the test compounds

The administration of Y-23684 and diazepam dose-dependently blocked convulsions triggered by bicuculline, pentylenetetrazol and MES in rats and mice (Table 2). However, these seizures were blocked in a divergent manner by Y-23684. Tonic extensions induced by bicuculline were antagonized by Y-23684 with an ED_{50} of 1.3 mg kg^{-1} in rats and 1.2 mg kg^{-1} in mice, whereas those triggered by MES

Table 1 Affinity of Y-23684 and diazepam for various neurotransmitter receptors in rat brain

Receptor	*^{3}H-ligand*	*Index* (nM)	*Brain tissue*	*Y-23684*	*Diazepam*
Benzodiazepine	Diazepam	Ki	Cerebral cortex	41	5.8
$GABA_A$	Muscimol	IC_{50}	Cerebral cortex	>100000	>100000
Dopamine D_2	Spiperone	IC_{50}	Striatum	>100000	>100000
Noradrenaline α_1	Prazosin	IC_{50}	Cerebral cortex	>100000	>100000
Noradrenaline α_2	Clonidine	IC_{50}	Cerebral cortex	>100000	>100000
5-HT_{1A}	8-OH-DPAT[a]	IC_{50}	Hippocampus	>100000	>100000
5-HT_2	Spiperone	IC_{50}	Cerebral cortex	>100000	>100000
Muscarine	QNB[b]	IC_{50}	Cerebral cortex	>100000	>100000

[a]8-OH-DPAT: 8-hydroxy-2-(di-n-propylamino)tetraline.
[b]QNB: 3-quinuclidyl benzilate.

Table 2 Anticonvulsive potency of Y-23684 and diazepam against bicuculline-, pentylenetetrazol- and maximal electrical shock (MES)-induced seizures

		ED_{50} (95% confidence limits) mg kg^{-1}, p.o.	
Convulsants	*Animals*	*Y-23684*	*Diazepam*
Bicuculline	Rats	1.3 (0.7–2.1)	13.3 (9.9–18.0)
	Mice	1.2 (0.8–1.9)	0.4 (0.2–0.5)
Pentylenetetrazol	Rats	15.6 (11.4–21.0)	12.8 (8.8–19.2)
	Mice	11.7 (7.4–17.0)	1.1 (0.8–1.6)
MES	Mice	20.5 (13.6–29.8)	1.7 (1.2–2.3)

Y-23684 was administered orally 3 h and 1 h before the test in rats and mice, respectively. Diazepam was administered orally 1 h before the test in both species. Results are shown as the ED_{50} values and their 95% confidence limits to prevent each convulsive response determined by probit analysis with 7–21 animals per group.

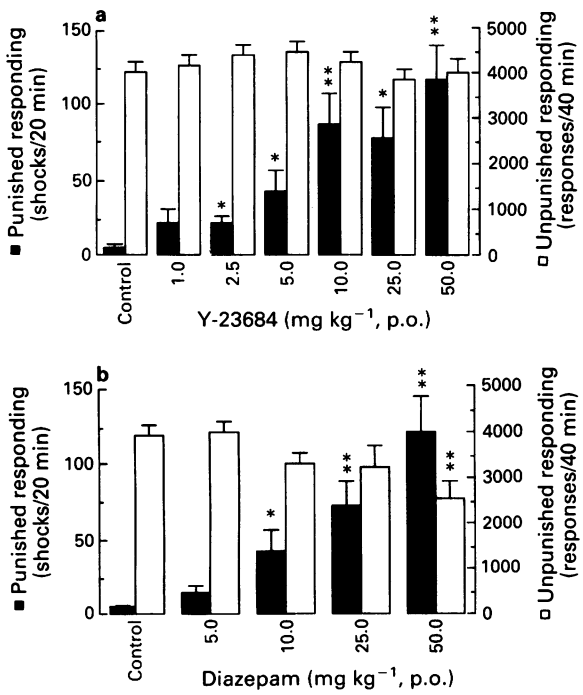

Figure 2 Effects of Y-23684 and diazepam in the rat Geller-Seifter test. Y-23684 and diazepam were administered orally 2.5 and 0.5 h before the test, respectively. Closed and open columns indicate punished and unpunished responding, respectively. Results are shown as the mean (with s.e.) of 10–11 animals per group. Significantly different from each control group $*P<0.05$; $**P<0.01$ (Aspin-Welch's t test).

were antagonized with an ED_{50} of 20.5 mg kg^{-1} in mice. Y-23684 also protected against lethality triggered by pentylenetetrazol with an ED_{50} of 15.6 mg kg^{-1} in rats and 11.7 mg kg^{-1} in mice. In contrast, the anticonvulsant profile of diazepam was characterized by nonselective antagonism of seizures triggered by these convulsants. In mice, convulsions induced by bicuculline, MES and pentylenetetrazol were antagonized with ED_{50}s of 0.4, 1.7 and 1.1 mg kg^{-1}, respectively. In rats, diazepam also blocked convulsions induced by bicuculline and pentylenetetrazol in a similar manner with ED_{50}s of 13.3 and 12.8 mg kg^{-1}, respectively.

Effects of the test compounds in the Geller-Seifter and water-lick conflict tests

In the Geller-Seifter test, Y-23684 and diazepam produced dose-dependent increases in punished responding, with significant effects occurring at 2.5 mg kg^{-1} for Y-23684 and 10 mg kg^{-1} for diazepam (Figure 2). Unpunished responding remained stable under Y-23684 (1–50 mg kg^{-1}), whereas diazepam at 50 mg kg^{-1} caused a large decrease in unpunished responding.

In the water-lick test, significant increases in licking were noted following the administration of Y-23684 (5–50 mg kg^{-1}) and diazepam (10 and 25 mg kg^{-1}) (Figure 3). However, the magnitude of the increase induced by Y-23684 was somehow less than that by diazepam.

Effects of the test compounds on social interaction behaviour

The administration of Y-23684 and diazepam dose-dependently increased social interaction with MEDs of 0.1 and 1.0 mg kg^{-1}, respectively (Figure 4). The effect of Y-23684 on social interaction was as efficacious as that of diazepam. There were no significant changes in locomotor activity throughout the dose-range of Y-23684, although diazepam at 25 mg kg^{-1}, (25 fold more than its MED on social interaction) produced an obvious sedative action that resulted in reduced interaction and line crossings.

Effects of the test compounds in the elevated plus-maze test

In the elevated plus-maze test, Y-23684 and diazepam increased the time spent on the end sections of open arms in a dose-related manner, with significant effects occurring at 0.05 mg kg^{-1} for Y-23684 and 0.5 mg kg^{-1} for diazepam (Figure 5).

Effects of the test compounds in the exploration (light/dark box) test

Y-23684 and diazepam produced an increase in exploratory behaviour (line crossings and exploratory rearings) in the light section of the test box accompanied by a decrease in exploratory behaviour in the dark section; also there was an increase in the amount of time spent in the light section (Figures 6 and 7). These changes attained significance for

three indices at 1.0 mg kg^{-1} of Y-23684 and 0.5 mg kg^{-1} of diazepam. However, at 10.0 mg kg^{-1} of diazepam, there appeared to be some evidence for the development of sedation indicated by a non-specific decrease in the behaviours measured, whereas Y-23684 did not show any similar signs up to 50 mg kg^{-1}.

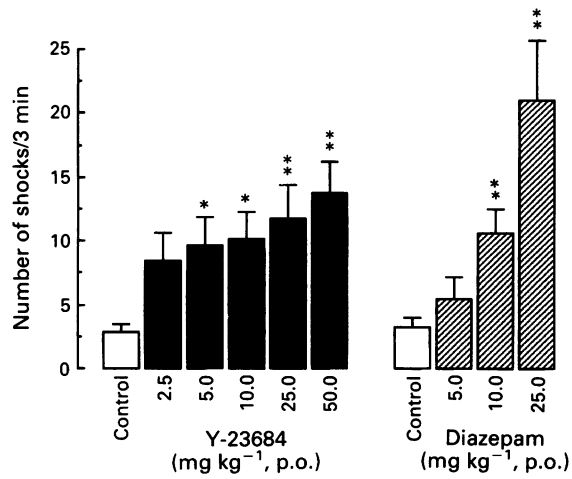

Figure 3 Effects of Y-23684 and diazepam in the water-lick conflict test. Y-23684 and diazepam were administered orally 3 and 1 h before the test, respectively. Results are shown as the mean with s.e. of 10–20 animals per group. Significantly different from each control group. *$P<0.05$; **$P<0.01$ (ANOVA followed by the LSD test).

Effects of the test compounds on rotarod performance and interaction with CNS depressants

Table 3 summarizes the potency of the test compounds in impairing rotarod performance and the loss of righting reflex after treatment with ethanol and hexobarbitone. In these tests, Y-23684 showed far less potent activity than diazepam in both rats and mice.

Discussion

In *in vitro* binding studies, Y-23684 showed a high and selective affinity for the BZR in the rat brain in spite of its non-BZ structure. Furthermore, it was reported previously that the potentiation of the GABA response by Y-23684 was less marked than that of conventional BZ-anxiolytics in frog sensory neurones *in vitro*, indicating its partial agonistic property on the BZR (Yakushiji *et al.*, 1990). The present paper describes the behavioural profile of Y-23684 in both various models of anxiety and in tests assessing adverse properties associated with classical BZs in comparison with those of diazepam.

The most frequently used paradigms for assessing the potential therapeutic efficacy of BZR agonists are anticonvulsant and anticonflict tests (Haefely *et al.*, 1990). In the seizure models in rodents presented here, the anticonvulsant profile of Y-23684 depended upon the convulsant: diazepam did not show such variation. Similar phenomena have been demonstrated previously with bretazenil and abecarnil which also possess partial agonistic properties on the BZR (Martin

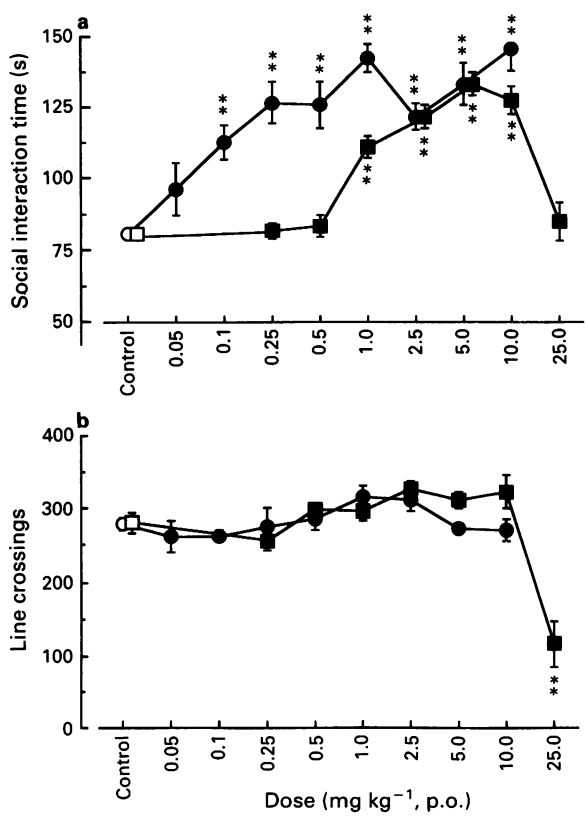

Figure 4 Effects of Y-23684 and diazepam on rat social interaction behaviour. Y-23684 and diazepam were administered orally 3 and 1 h before the test, respectively. Points indicate mean ± s.e. of 5–10 pairs of animals per group treated with Y-23684 (●) and diazepam (■), respectively. Time of social interaction behaviour (a) and the number of line crossings (b) were measured. Significantly different from each control group. *$P<0.05$; **$P<0.01$ (ANOVA followed by Dunnett's t test).

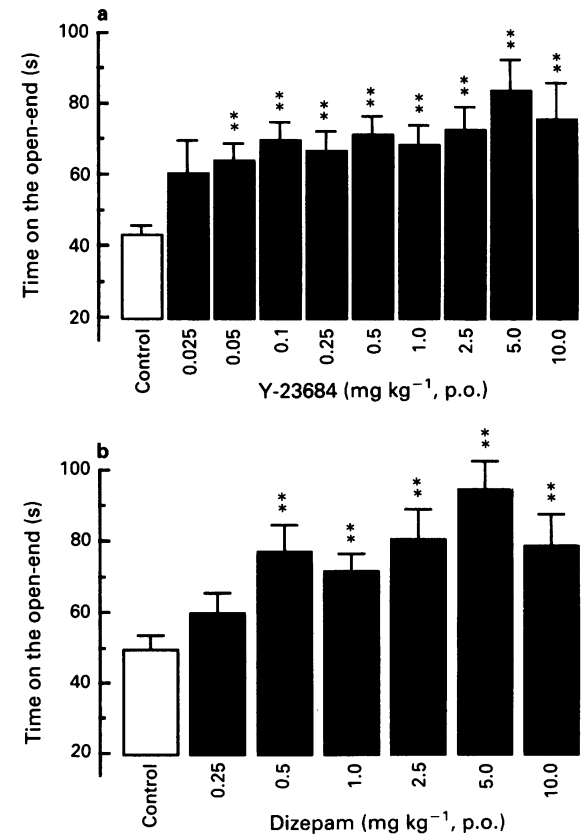

Figure 5 Effects of Y-23684 (a) and diazepam (b) in the elevated plus-maze test. The time on the open-end was measured. Y-23684 and diazepam were administered orally 3 and 1 h before the test, respectively. Results are shown as the mean with s.e. of 16–24 animals per group. Significantly different from each control group. *$P<0.05$; **$P<0.01$ (Kruskal-Wallis ANOVA followed by Mann-Whitney's U-test).

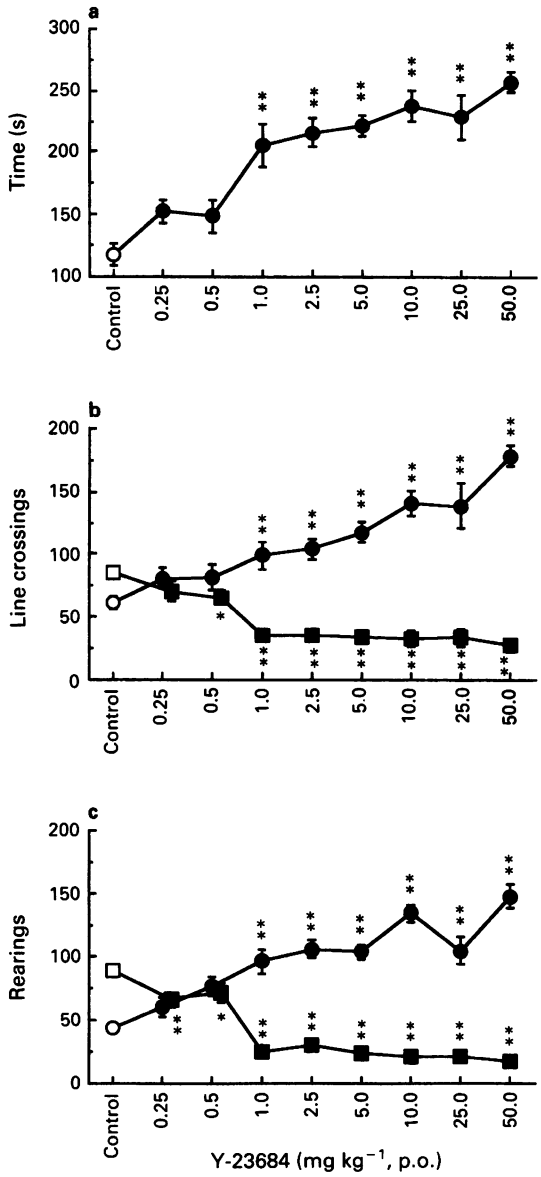

Figure 6 Effects of Y-23684 in the mouse exploration (light/dark box) test. Y-23684 was administered orally 1 h before the test. Values in the light (●) and dark (■) compartments are shown. Time spent in the light compartment (a), the numbers of line crossings (b) and the number of exploratory rearings (c) were measured. Results are shown as the mean ± s.e. of 8–24 animals per group. Significantly different from each control group. $*P<0.05$; $**P<0.01$ (ANOVA followed by Dunnett's t test).

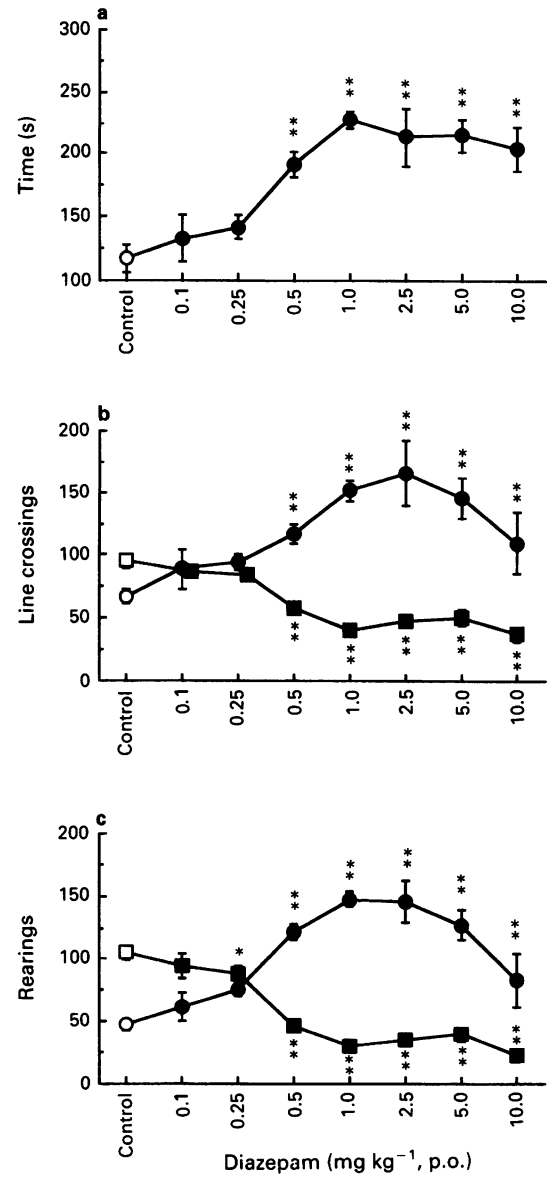

Figure 7 Effects of diazepam in the mouse exploration (light/dark box) test. Diazepam was administered orally 1 h before the test. Values in the light (●) and dark (■) compartments are indicated. Time spent in the light compartment (a), the numbers of line crossings (b) and the number of exploratory rearings (c) were measured. Results are shown as the mean ± s.e. of 8–16 animals per group. Significantly different from each control group: $*P<0.05$; $**P<0.01$ (ANOVA followed by Dunnett's t test).

et al., 1988; Turski *et al.*, 1990). The differences between Y-23684 and diazepam may account for the possibility that they and/or convulsants act on heterogenic domains of the BZR/GABA-chloride channel ionophore complexes in different ways. This possibility appears to be supported in part by previous findings that bicuculline is a $GABA_A$ receptor antagonist whilst pentylenetetrazol acts on the BZR and/or the *t*-butylbicyclophosphorothionate (TBPS) binding site on the complexes (Rehavi *et al.*, 1982; Bowery *et al.*, 1983; Ramanjaneyulu & Ticku, 1984). One might argue that the compounds show variations in the BZR occupancy in different brain areas mediating each convulsive response and/or its spreading. These inferences should be proved with biochemical and electrophysiological techniques.

Y-23684 showed antipunishment properties in the rat Geller-Seifter and water-lick conflict tests; it was effective at doses 2 to 4 times lower than diazepam. In contrast to diazepam, Y-23684 did not alter unpunished responding in the Geller-Seifter paradigm at any of the doses tested. On the other hand, it induced a consistent effect in the water-lick conflict test, but its activity was weaker than diazepam. The reason for this difference in activity between Y-23684 and diazepam is unclear. However, since these classical conflict paradigms were originally optimized for conventional BZ-anxiolytics (BZR full agonists), what is considered to be an 'antipunishment' activity may be composed of any combination of antipunishment, sedative, myorelaxant, amnesic or anticonvulsant elements (Singh *et al.*, 1991). In addition, as food or water-deprived rats were exposed to conflict situations involving rewards and punishment in these paradigms, motivational changes produced by the test compounds may interfere with detection of their antipunishment activity. Previous studies have demonstrated the ability of diazepam to increase food or water-intake under non-conflict situations

Table 3 Effect of Y-23684 and diazepam on rotarod performance and potentiation of ethanol- and hexobarbitone-induced loss of righting reflexes

Tests	Animals	ED_{50} (95% confidence limits) mg kg^{-1}, p.o. Y-23684	Diazepam
Rotarod	Rats	$\geqslant 100$ (5/10)[a]	8.8 (6.5–12.2)
	Mice	100.1 (68.6–147.8)	1.7 (1.2–2.6)
Ethanol potentiation	Rats	>250 (5/14)[a]	6.1 (3.9–10.0)
	Mice	$\geqslant 500$ (7/14)[a]	3.4 (2.3–4.6)
Hexobarbitone potentiation	Rats	$\geqslant 250$ (7/14)[a]	12.4 (8.7–20.0)
	Mice	80.3 (44.1–138.7)	2.6 (1.4–3.9)

Y-23684 was administered orally 3 h and 1 h before the test in rats and mice, respectively. Diazepam was administered orally 1 h before the test in both species. Results are shown as the ED_{50} values and their 95% confidence limits to impair rotarod performance and potentiate loss of the righting reflex after administration of ethanol or hexobarbitone determined by probit analysis with 7–14 animals per group. [a]If an ED_{50} could not been obtained throughout the tested doses, each result at the indicated maximum dose is shown as (the number of animals which showed positive effects/the number of animals used).

that leads to motivational changes (Johnson, 1978; Shimizu *et al.*, 1987). In contrast, we failed to detect any changes in free drinking with Y-23684 when measuring the volume of water consumed from a spout during a 3 min test session under a non-conflict situation in a pilot study (data not shown). Therefore, we conclude that Y-23684, which displays little hyperdipsic effect, should show a different profile from diazepam in the water-lick conflict test. This view is supported by the evidence obtained in other models of anxiety which did not involve water- or food-deprivation and electrical shocks as discussed below.

Recently, not only conventional conflict models but behavioural suppression induced by exposing animals to aversive environmental stimuli have been used extensively to assess the anxiolytic profile of known clinically active anxiolytic agents (Chopin & Briley, 1987; File, 1991). However, there have as yet, been few systematic comparisons of the anxiolytic actions of BZR full and partial agonists in these paradigms.

Firstly, Y-23684 and diazepam increased social interaction relative to controls under conditions of maximal environmental suppression (high light, unfamiliar condition) in the present study. There were no increases in locomotion in groups displaying enhanced social interaction, suggesting that the two measures are independent. This would rule out the possibility that the increased social interaction was the result of a direct stimulatory action of these compounds (Higgins *et al.*, 1988; 1992). Importantly, the increased social interaction induced by Y-23684 was maintained over a wide dose range (100 fold) without loss in efficacy and sedative action, highlighting a distinctive 'anxioselective' anxiolytic property of Y-23684.

Secondly, on the elevated maze used in the present paradigm, the tendency of rats, which had been housed under dim red lights, to explore their environments conflicted with the inherent tendency of these rats to avoid brightly lit and the end parts of the open arms. The present result is consistent with the idea that Y-23684 and diazepam possess anxiolytic activity during this aversive state.

Thus, in these two rat models of anxiety, Y-23684 was as efficacious and ten fold more potent than diazepam. Furthermore, it is important to mention that Y-23684 and diazepam displayed anxiolytic activity at lower doses in these paradigms than those in classical conflict models, Geller-Seifter and water-lick tests.

Finally, the measurement of changes in exploration of mice as measured in a light and dark test box would appear to offer a particularly simple model of anxiety (Crawley & Goodwin, 1980), the method being based on the observation that whilst rodents tend to explore a novel environment, open fields appear to have aversive properties which may inhibit their exploratory behaviour (Costall *et al.*, 1989a). As a consequence, Y-23684 and diazepam were shown to reinstate suppressed behaviours. However, Y-23684 did not cause any sedative effects in contrast to diazepam. Again in this study, the magnitude of the anxiolytic effect of Y-23684 was not less than that of diazepam. The fact that diazepam showed a more potent anxiolytic action than Y-23684 in this paradigm can be explained by an *ex vivo* [^{3}H]-diazepam binding study in mice. The inhibition of specific binding by orally administered diazepam was more potent than that by Y-23684, whereas this trend was reversed in rats (unpublished observations).

This 'anxioselective' characteristic of Y-23684 is supported in tests assessing unwanted side-effects. Impairment in the rotarod test and potentiation of CNS depressants by Y-23684 were 11–147 times weaker than diazepam. This shows a marked separation of the anxiolytic and undesired side-effects of Y-23684 in contrast to diazepam.

An important mechanistic explanation of the anxioselective profile of Y-23684 comes from a recent finding in an electrophysiological study with mammalian CNS neurones (Yakushiji *et al.*, 1993). In that study, each BZR subtype-density contributed little to efficacy of the augmentation of the GABA-induced chloride currents by Y-23684 in acutely dissociated rat cerebeller Purkinje (CPJ) neurones (BZ_1 subtype predominant) and spinal ventral horn (SVH) neurones (BZ_2 subtype predominant), whereas the augmentation by diazepam depended upon the BZ_2 receptor-density. Indeed, the augmentation of the GABA response by Y-23684 was observed to be as efficacious in CPJ neurones but to be less in SVH neurones than diazepam which displayed the more marked enhancement in the latter neurones. This characterization relates to lower intrinsic activities of Y-23684 than diazepam, a conventional BZR full agonist, at the BZ_2 receptors even at their highly 'reserved' phase (Hoyer & Boddeke, 1993). Although the functional specificity of BZR multiplicity is unsettled, one possible way of distinguishing the anxioselective profile of Y-23684 from diazepam arises from the difference in intrinsic activities at the BZ_2 sites.

In conclusion, the present studies demonstrate that Y-23684, a BZR partial agonist, produced potent anxiolytic effects in several rodent models of anxiety. Furthermore, Y-23684 was shown to improve the side-effect profile seen in a conventional BZ, diazepam. Therefore, Y-23684 is predicted to be a potent anxioselective anxiolytic agent that exhibits considerable advantages over conventional BZs in man.

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