0026-895X/88/020124-05$02.00/0

MOLECULAR PHARMACOLOGY, 34:124-128

In Vivo Binding of β-Carbolines in Mice: Regional Differences and Correlation of Occupancy to Pharmacological Effects

M.-C. POTIER, L. PRADO DE CARVALHO, R. H. DODD, R. BESSELIEVRE, and J. ROSSIER

Laboratoire de Physiologie Nerveuse (M.-C.P., L.P.C., J.R.) and Institut de Chimie des Substances Naturelles (R.H.D., R.B.), CNRS, 91190 Gif-sur-Yvette, France

Received November 10, 1987; Accepted May 16, 1988

SUMMARY

β-Carbolines are competitive ligands of central type benzodiazepine receptors and have been reported to display either agonist, inverse agonist, or antagonist activities *in vivo*. We studied the *in vivo* inhibition of [^{3}H]Ro 15-1788 binding in mice by various β-carbolines of different pharmacological profiles and found that they were all more potent in the cerebellum than in the cortex and hippocampus; their ID_{50} values (dose inhibiting 50% of the specific binding of [^{3}H]Ro 15-1788) were 3 to 7 times lower in the cerebellum than in the hippocampus. The ID_{50} of the triazolopyridazine CL 218,872 was 2.3 times lower in the cerebellum than in the hippocampus. Thus, regional differences do not seem to explain pharmacological profile.

Correlation of receptor occupancy with pharmacological effects showed that high receptor occupancy (40%) was needed to obtain the convulsant effects of the inverse agonist methyl-β-carboline-3-carboxylate whereas intermediate receptor occupancy (30%) led to the proconflict effects and very low receptor occupancy (<5%) to the facilitating effects in learning and memory tasks. We also found that the selective antagonist of the sedative effects of benzodiazepines 3-(methoxycarbonyl)-amino-β-carboline, even at high doses (20 mg/kg), did not occupy more than 70% of the benzodiazepine receptors.

Specific binding sites for the benzodiazepine type of minor tranquillizers have been demonstrated in the central nervous system of vertebrates (1–3) and much evidence suggests that these binding sites are the receptors responsible for mediating the pharmacological and clinical effects of benzodiazepines.

New types of ligands of molecular structure different from benzodiazepine have been discovered that bind to the benzodiazepine receptor with high affinity. Among these are the 3-carboxy-β-carbolines. *In vivo*, β-CCE (4) and β-CCM (5) were found to be inverse agonists at the benzodiazepine receptor, with pharmacological properties opposite to those of benzodiazepine: β-CCE is proconvulsant (6), β-CCM is convulsant (7) and anxiogenic (8). Other β-carbolines were shown to be agonists (ZK 93423) (9) or antagonists, such as β-CCP (10) and β-CMC, a selective antagonist of the sedative effects of diazepam (11).

In vitro, some of these β-carbolines (β-CCM, β-CCE, and β-CCP) are more potent in inhibiting the binding of tritiated benzodiazepine from the cerebellar binding sites than from the cortical or the hippocampal ones (12–15). Similar selectivity *in vivo* could be the basis for the different pharmacological profiles of these molecules.

This work was supported by Grant 84185 from DRET and by PIRMED. M.C.P. was supported by a fellowship from DRET and CNRS.

Thus, in the present report, the *in vivo* interactions of several β-carbolines with benzodiazepine binding sites in the cerebellum, cortex, and hippocampus were studied after labeling of the benzodiazepine receptors with the tritiated antagonist [^{3}H]Ro 15-1788 (16). We compared the *in vivo* inhibition of [^{3}H]Ro 15-1788 binding by β-carbolines (β-CCM, β-CCE, β-CCP, β-CMC, and ZK 93423) with the inhibition by the triazolopyridazine CL 218,872 and by diazepam in the three brain regions. We find that *in vivo*, in spite of differences in their pharmacological profiles, all the β-carbolines tested are more potent in inhibiting [^{3}H]Ro 15-1788 binding in the cerebellum than in the cortex or hippocampus.

Materials and Methods

Animals. Male Swiss mice (20–25 g) were obtained from Iffa Credo rearing center (L'Arbresle, France) and housed for a few days before the beginning of the experiments, with a day-night cycle of 12-12 hr.

Drugs. β-CCM, β-CCE, and β-CMC were synthesized by one of us (17). They were dissolved in 0.1 N HCl (100 μl/mg) and diluted to volume with saline. ZK 93423 was a gift from Schering and CL 218,872 from Lederle. The latter two compounds were suspended in saline with a drop of Tween 80.

Diazepam (Valium) and flunitrazepam were kindly provided by Hoffman-La Roche (Basel, Switzerland). Flunitrazepam was suspended in saline with a drop of Tween 80, and Valium was diluted in saline.

ABBREVIATIONS: β-CCE, ethyl-β-carboline-3-carboxylate; β-CCM, methyl-β-carboline-3-carboxylate; ZK 93423, ethyl-6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate; β-CCP, propyl-β-carboline-3-carboxylate; β-CMC, 3-(methoxycarbonyl)-amino-β-carboline; CL 218,872, 3-methyl-6-(3-(trifluoromethyl)phenyl)-1,2,4-triazolo(4,3-b)pyridazine; GABA, γ-aminobutyric acid; IV, intravenous; IP, intraperitoneal; SC, subcutaneous.

[^{3}H]Ro 15-1788 (87 Ci/mmol) and [^{3}H]β-CCE (79.4 Ci/mmol) were from Dupont NEN (Boston, MA). [^{3}H]β-CMC (79.25 Ci/mmol) was prepared by R. Besselièvre at the Commissariat à l'Energie Atomique (Saclay, France); it was tritiated on the methyl ester by a Curtius reaction of the acylazide in the presence of tritiated methanol.

In vivo binding experiments. *In vivo* labeling of the mouse brain benzodiazepine receptors was performed as previously described (16). Briefly, mice were injected IV with [^{3}H]Ro 15-1788 (50 μCi/kg) and decapitated 3 min later, a time corresponding to maximal radioactivity in the brain (16). Their brains were rapidly excised and dissected on ice. Cortex from one hemisphere, whole cerebellum, and both hippocampi were homogenized in approximately 30 volumes (3 ml for cerebellum and cortex and 1.5 ml for hippocampus) of ice-cold 50 mM Tris·HCl buffer, pH 7.4, using a Kinematica Polytron (type PT 10/35) for 5 sec, at half maximal speed.

Aliquots of 600 μl of the homogenate were immediately filtered through Whatman glass fiber filters (GF/B, 2.5 cm) and the filters were rinsed with two 5-ml portions of ice-cold buffer. The filters were transferred into vials and after addition of 10 ml aqueous counting scintillant (Amersham, France, Les Ulis, France) were counted in a Rack-β-2 LKB counter. The radioactivity retained on the filters represented the radioactivity bound to membranes.

The total radioactivity (bound plus free) present in the brain was determined by counting 200-μl aliquots of the homogenates without filtering. Nonspecific binding was determined in mice pretreated IP with flunitrazepam (10 mg/kg) 30 min before death and represented less than 5% of the binding determined in mice injected with [^{3}H]Ro 15-1788 only.

To study the effects of drugs on the *in vivo* binding of [^{3}H]Ro 15-1788, mice were pretreated with various doses of β-CCM, β-CCE, β-CMC, β-CCP, ZK 93423, and diazepam before the IV injection of [^{3}H]Ro 15-1788. Unless otherwise stated, drugs were injected SC 10 min before death. CL 218,872 was injected IP 30 min before death. These time intervals were chosen on the basis of time required for appearance of pharmacological effects.

For each dose of unlabeled drug injected, the membrane-bound radioactivity was measured and specific binding was calculated by subtracting the nonspecific binding. Results were expressed as percentage of the specific binding determined in mice injected with radioactive ligand only.

For each drug, inhibition curves were obtained from four or five doses (four mice per dose). ID_{50} values (dose inhibiting 50% of the specific binding of [^{3}H]Ro 15-1788) were calculated from a linear regression analysis of $\log\left(\frac{\%}{100-\%}\right)$ on log (dose).

For each drug tested, confidence intervals at 95% of log (ID_{50}) in the three brain regions studied were calculated by a linear regression analysis (18) on log-logit transformations ($p < 0.05$). Tween 80 alone had no effect on [^{3}H]Ro 15-1788 binding.

Absorption and distribution of [^{3}H]β-CMC and [^{3}H]β-CCE. Groups of two or four mice were injected with [^{3}H]β-CMC or [^{3}H]β-CCE via the tail vein (200 μCi/kg). Three min after the injection of [^{3}H]β-CCE, and 2 or 10 min after the injection of [^{3}H]β-CMC, mice were decapitated and their brains were excised, dissected, and homogenized as described above. Aliquots (200 μl) of the homogenate (nonfiltered) were counted by liquid scintillation.

Results

In a first set of experiments, β-CCM and β-CMC were injected SC 7 min before the IV injection of [^{3}H]Ro 15-1788. A dose-dependent inhibition of [^{3}H]Ro 15-1788 binding was observed in the three brain regions studied for both drugs. However, displacement curves showed regional differences. Fig. 1 illustrates results obtained with β-CMC: high dose (20 mg/kg SC) of β-CMC inhibited nearly 70% of [^{3}H]Ro 15-1788 in the cortex and cerebellum but only 50% in the hippocampus. Indeed, the doses of β-CCM and β-CMC inhibiting 50% of the specific binding of [^{3}H]Ro 15-1788 (ID_{50} values) were found to be lower in the cerebellum than those in the cortex, which were in turn lower than those in the hippocampus (Table 1). Results are also expressed as the ratio of ID_{50} values in the hippocampus and cerebellum (Table 1). High ratios suggest cerebellar preference. Fig. 2 shows the log-logit plots of inhibition curves with β-CCM and β-CMC in the cortex.

In the above described experiments, when β-CCM (5 mg/kg) was injected 10 min before death (i.e., 7 min before administration of [^{3}H]Ro 15-1788), it produced tonic-clonic seizures in

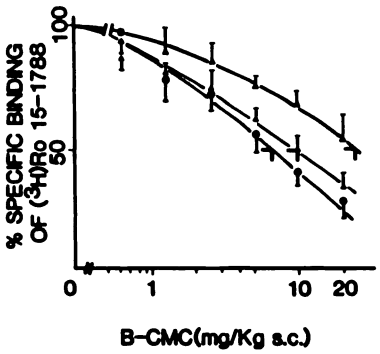

Fig. 1. Inhibition curve of [^{3}H]Ro 15-1788 binding by β-CMC injected SC 7 min before [^{3}H]Ro 15-1788 and 10 min before sacrifice in the cerebellum (●), cortex (▲), and hippocampus (△).

TABLE 1

ID_{50} values of β-carbolines, benzodiazepines, and a triazolopyridazine with respect to [^{3}H]Ro 15-1788 binding in the cerebellum, cortex, and hippocampus

Drug injected	ID_{50} values for [^{3}H]Ro 15-1788			$\frac{ID_{50}\text{ hippocampus}}{ID_{50}\text{ cerebellum}}$
	Cerebellum	Cortex	Hippocampus	
			mg/kg	
β-CCE	4.8	8.4	32.6	6.8[a]
β-CCM	0.72	1.6	4.8	6.7[a]
β-CMC	6.8	9.8	25.3	3.7[a]
ZK 93423	7.8	19.4	24.6	3.1[a]
CL 218,872	12	19	28	2.3[a]
Diazepam (30 min)	4.41	8.3	8.8	2.0
Diazepam (10 min)	12.7	25.5	20.9	1.6
Ro 15-1788[b]	0.16	0.22	0.26	1.12
Flunitrazepam[b]	0.48	0.56	0.42	0.87

[a] log (ID_{50}) values did not overlap in cerebellum and hippocampus ($p < 0.05$).
[b] From Potier *et al.* (16). Correlation coefficients for log-logit plots were superior to 0.9.

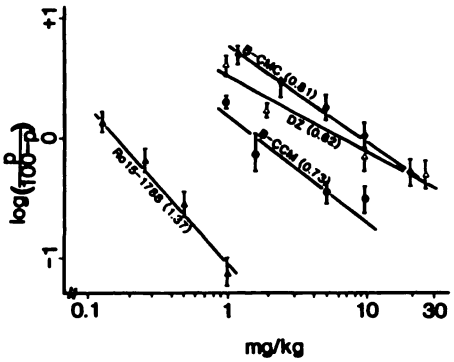

Fig. 2. Log-logit plots of the inhibition of [^{3}H]Ro 15-1788 binding by diazepam (△), β-CCM (●), β-CMC (♦), and Ro 15-1788 (▲) in the cortex. *Numbers in parenthesis* are the negative slopes of the regression lines.

50% of the mice with short median latencies (2–3 min after injection) and it induced 74, 72, and 55% inhibition of specific binding of [^{3}H]Ro 15-1788 in the cerebellum, cortex, and hippocampus, respectively. When the same dose of β-CCM (5 mg/kg) was administered 2 min before death (i.e., 1 min after the IV administration of [^{3}H]Ro 15-1788), the time interval was not sufficient for the development of convulsions, and only 60, 35, and 30% of the specific binding of [^{3}H]Ro 15-1788 was inhibited in the cerebellum, cortex, and hippocampus, respectively.

Other β-carbolines were also tested for their potencies in inhibiting [^{3}H]Ro 15-1788 binding in the three brain regions. Mice were pretreated with various doses of β-CCE or ZK 93423 SC 10 min before death. The ID_{50} values (Table 1) were, as with β-CCM and β-CMC, also lower in the cerebellum than in the cortex or hippocampus. The ratio of ID_{50} values in the hippocampus and the cerebellum was higher for β-CCE (6.8) than for ZK 93423 (3.1).

The preferential inhibition in the cerebellum of [^{3}H]Ro 15-1788 binding by β-CMC or by β-CCE was not due to a heterogeneous distribution of these drugs in the brain. Thus, 2 or 10 min after the IV injection of [^{3}H]β-CMC (200 μCi/kg), total radioactivity in homogenates of the three brain regions were not significantly different ($p \leq 0.05$), 254.2 and 282.9 pCi/mg of wet tissue in the cerebellum, 262.8 and 254.7 in the cortex, and 293.7 and 263.1 in the hippocampus 2 min after the injection, and 55.1 ± 9 in the cerebellum, 53.2 ± 8.5 in the cortex, and 60.6 ± 8.6 in the hippocampus ($n = 3$) 10 min after the injection. Similarly, 2 min after the injection of [^{3}H]β-CCE (200 μCi/kg), total radioactivity in the cerebellar and cortical homogenates were not significantly different ($p \leq 0.05$), 738.6 ± 47.7 pCi/mg of tissue in the cerebellum and 787.8 ± 63.8 in the cortex ($n = 4$).

β-CCP injected SC at doses lower than 10 mg/kg 10 min before sacrifice did not produce any detectable inhibition of [^{3}H]Ro 15-1788 binding. At 10 mg/kg, 10% inhibition was found only in the cerebellum and, at 30 mg/kg, higher inhibition was found in the cerebellum (35%) than in the cortex (13%) and hippocampus (10%).

Apparent differences in inhibition curves in the various brain regions were also observed with one benzodiazepine tested. When diazepam was injected SC 30 min or 10 min before death the ID_{50} values seemed lower in the cerebellum than in the hippocampus or cortex but the ratio of the ID_{50} values was not as high as with β-carbolines (Table 1). Moreover, confidence intervals of log(ID_{50}) in the three brain regions, calculated by a linear regression analysis (see Materials and Methods), were overlapping, which was not the case for β-carbolines.

CL 218,872, a triazolopyridazine claimed to be a selective anxiolytic, is known to have a higher affinity for the cerebellar than for the hippocampal binding sites *in vitro* (19). In *in vivo* binding studies, when injected IP 30 min before death, CL 218,872 dose dependently inhibited the binding of [^{3}H]Ro 15-1788 but, as with β-carbolines and diazepam, ID_{50} values were higher in the hippocampus and cortex than in the cerebellum (Table 1). In addition, confidence intervals of log(ID_{50}) in cerebellum and hippocampus were not overlapping (see Materials and Methods).

Discussion

***In vivo* molecular interactions of β-carbolines and benzodiazepines with the benzodiazepine receptors.** The β-carbolines used in this study, whether agonist (ZK 93423), inverse agonists (β-CCM and β-CCE), or antagonists (β-CMC and β-CCP), as well as the triazolopyridazine CL 218,872, all showed lower ID_{50} values in the cerebellum than in the cortex and hippocampus. Similar results have already been observed with β-CCE in mice (20, 21) and with CL 218,872 in rats (22). In contrast, this regional difference was not observed with flunitrazepam or with Ro 15-1788 (Table 1).

Preferential inhibition of the *in vivo* binding of [^{3}H]Ro 15-1788 by β-carbolines and CL 218,872 in the cerebellum could be due to different distribution and penetration of these compounds in brain or to different molecular interactions at the receptor protein level. The second explanation seems more likely because [^{3}H]β-CMC and [^{3}H]β-CCE (or their radioactive metabolites) were distributed uniformly in the three brain regions studied (cerebellum, cortex, and hippocampus). Moreover, pretreatment of mice with high doses (10 to 30 mg/kg) of β-CCM, β-CMC, β-CCE, ZK 93423, or CL 218,872 did not alter significantly ($p \leq 0.05$) the distribution of the free (unbound) radioactivity in the brain, as compared with mice injected with radioactive ligand only.

The possible existence of two different benzodiazepine receptors (BZ_1 and BZ_2) has been extensively discussed (12–15, 19); BZ_1 is the only type present in cerebellum, whereas cortex contains 80% of type 1 and 20% of type 2, and hippocampus contains 50% of each type. *In vitro*, most of the β-carbolines (β-CCE, β-CCP, and β-CCM) and the triazolopyridazine CL 218,872 are selective for the cerebellar over the hippocampal receptors (12–15, 19). In our *in vivo* binding studies, β-carbolines and CL 218,872 have lower ID_{50} values in cerebellum than those in cortex, which were in turn lower than those in hippocampus. Thus, the differences displayed in the *in vivo* binding of β-carbolines and CL 218,872 could be due to the selectivity of these molecules for the BZ_1 receptor type. Because β-carboline agonist, inverse agonists, and antagonists and CL 218,872 all have higher potency in inhibiting [^{3}H]Ro 15-1788 binding in the cerebellum *in vivo*, this selectivity for the cerebellum cannot account for the inverse agonist pharmacological profiles of some of the β-carbolines (β-CCM and β-CCE) or for the selective antagonist properties of β-CMC (11).

***In vivo* occupancy of benzodiazepine receptors: correlation with pharmacological effects.** Correlations of benzodiazepine receptor occupancy to pharmacological effect have already been established (23–27). However, most of the [^{3}H] benzodiazepine inhibition studies were not performed at the time coinciding with the onset of the pharmacological effects of the benzodiazepine.

Previous experiments performed in this laboratory have shown that the pharmacological effects displayed by both β-CCM and diazepam depend on the doses administered. For instance, β-CCM at 0.2 mg/kg has facilitating effects in learning and memory tasks (28) whereas higher doses (1 mg/kg) are necessary for inducing proconflict effects (8) and still higher doses (5–10 mg/kg) for inducing convulsant effects (7). In direct contrast, diazepam is anticonvulsant (against convulsions induced by pentylenetetrazol) at low doses (0.2 mg/kg) (29), displays anticonflict effects at a higher dose (1 mg/kg) (8), and impairs learning and memory tasks at 1.5 mg/kg (28). To help to elucidate this phenomenon, receptor occupancies by β-CCM and diazepam were measured at the time of onset of the different pharmacological effects (Table 2). It was demon-

TABLE 2

Correlation of *in vivo* benzodiazepine receptor occupancy by an agonist (diazepam) and an inverse agonist (β-CCM) with their pharmacological effects

Agonist (diazepam) effects	% of benzodiazepine receptor occupancy	Inverse agonist (β-CCM) effects
	35–55	Convulsant[b]
Ataxic[a]	30–40	
	15–50	Proconflict[c]
Impairment of learning[d]	10–20	
Anticonflict[c]	5–15	
Anticonvulsant[e]	<5	Facilitation of learning[d]

[a] Rotarod test, 2 mg/kg (7).
[b] 5 mg/kg (7).
[c] Conflict test, 1 mg/kg (8).
[d] Learning and memory tasks, 1.5 mg/kg for diazepam and 0.2 mg/kg for β-CCM (28).
[e] At 0.2 mg/kg against convulsions induced by pentylenetetrazol (29).

strated that β-CCM, at its ED_{50} for convulsions (5 mg/kg) occupied 35–55% of benzodiazepine receptors; at its proconflict dose (1 mg/kg) (8), β-CCM occupied 15–50% of receptors, and its facilitating effects in learning and memory tasks (28) appeared at very low receptor occupancy, <5% (exact values are not given because of limits to the sensitivity of the method). It was also demonstrated that an anticonvulsant (anti-pentylenetetrazol) dose of diazepam in mice (0.2 mg/kg) (29) corresponded to a very low level of benzodiazepine receptor occupancy (<5%), an anticonflict dose of diazepam (1 mg/kg) (8) to 5–15% occupancy, and the impairment in learning and memory tasks (1.5 mg/kg) (28) to 10–20% receptor occupancy. To obtain the sedative-ataxic effects of diazepam (2 mg/kg) (7), an even higher occupancy was needed (30–40%). It is thus clear that for both the agonist diazepam and the inverse agonist β-CCM, the progression of doses that induces different pharmacological effects correlates with progressive levels of receptor occupancy.

It is also noteworthy that all the known pharmacological effects of both the agonist and the inverse agonist can be observed when less than 50% of the benzodiazepine receptors are occupied and that some of these effects are evident at even less than 5% occupancy. Thus, very low benzodiazepine receptor occupancy may be sufficient to modulate GABAergic transmission, known to be facilitated by agonists and impaired by inverse agonists. In the case of opiate receptors, analgesic effects also correspond to a small percentage of receptor occupancy (2%) (30).

Relation between the slope of the inhibition curve and pharmacological effects. The slope of the inhibition curve of [^{3}H]Ro 15-1788 binding by a benzodiazepine receptor ligand plotted in log-logit may have some bearing on its pharmacological actions (Fig. 2). Shallow slopes reflect slow progression of receptor occupancy by increasing doses of the inhibitor. On the contrary, steep slopes reflect rapid occupation of receptor. Because the pharmacological effects of a ligand of a given intrinsic activity (agonist, inverse agonist, or antagonist) partially depend on the level of receptor occupancy, the slope could be an estimation of the therapeutic index, which is the margin of safety or selectivity for one effect among a spectrum of effects (31).

If that is the case, a benzodiazepine receptor agonist showing a shallow slope should be able to clearly dissociate the anticonvulsant-anxiolytic effects from the sedative-amnesic effects depending on the dose used. This would be explained by the fact that anticonvulsant-anxiolytic effects appear at 10% receptor occupancy, but large dose increases are necessary to produce the level of receptor occupancy (around 30%) required to exert the sedative-amnesic effects. A benzodiazepine agonist with steep slope would produce all the spectrum of effects at the same or very similar dose.

In the same way as the agonists, a benzodiazepine receptor antagonist showing a steep slope would be able to antagonize all the effects of benzodiazepine agonists within a very restricted dose range. An example is given with Ro 15-1788, which is known to antagonize all the effects of benzodiazepines (32); nearly 100% benzodiazepine receptor occupancy was obtained after 1 mg/kg and 50% was obtained after 0.16 mg/kg (Fig. 2).

According to this interpretation, increasing doses of a benzodiazepine receptor antagonist with a shallow slope would progressively antagonize the various effects of benzodiazepine agonists. In addition, antagonists that, even at high doses, do not occupy 100% of receptors, would only block the effects requiring high levels of occupancy (sedation). β-CMC is an example of this type of selective antagonist; it has a shallower slope than Ro 15-1788, at doses up to 20 mg/kg SC it does not occupy more than 70% of benzodiazepine receptors, and it antagonizes the sedative effects of benzodiazepines but not their anxiolytic or anticonvulsant effects (11).

Finally, according to the above discussed interpretation of inhibition curves *in vivo*, an inverse agonist showing a shallow slope for inhibition of *in vivo* binding would be able at low doses to enhance performance in learning and memory tasks without being anxiogenic and convulsant; a higher dose would have to be administered to obtain the high level of receptor occupation required for the induction of anxiogenic and convulsant effects.

Acknowledgments

We are very grateful to C. L. Brown for useful discussions and we thank B. Lambolez and P. Bochet for reading the manuscript.

References

1. Braestrup, C., and R. F. Squires. Specific benzodiazepine receptors in rat brain characterized by high affinity [^{3}H]diazepam binding. *Proc. Natl. Acad. Sci. USA* **74**:3805–3809 (1977).
2. Mohler, H., M. K. Battersby, and J. G. Richards. Benzodiazepine receptor: demonstration in the central nervous system. *Science (Wash. D. C.)* **198**:849–851 (1977).
3. Bosmann, H. B., K. R. Case, and P. Distefano. Diazepam receptor characterization: specific binding of a benzodiazepine to macromolecules in various areas of the rat brain. *FEBS Lett.* **82**:368–372 (1977).
4. Braestrup, C., M. Nielsen, and C. E. Olsen. Urinary and brain β-carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors. *Proc. Natl. Acad. Sci. USA* **77**:2288–2292 (1980).
5. Rommelspacher, H., C. Nanz, H. A. Borbe, K. J. Fehske, W. E. Muller, and U. Wollert. 1-methyl-β-carboline (harmane), a potent endogenous inhibitor of benzodiazepine receptor binding. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **314**:97–100 (1980).
6. Tenen, S. S., and J. D. Hirsch. β-Carboline-3-carboxylic acid ethyl ester antagonizes diazepam activity. *Nature (Lond.)* **288**:609–610 (1980).
7. Prado de Carvalho, L., G. Grecksch, E. A. Cavalheiro, R. H. Dodd, G. Chapouthier, and J. Rossier. Characterization of convulsions induced by methyl β-carboline-3-carboxylate in mice. *Eur. J. Pharmacol.* **103**:287–293 (1984).
8. Prado de Carvalho, L., G. Grecksch, G. Chapouthier, and J. Rossier. Anxiogenic and non-anxiogenic benzodiazepine antagonists. *Nature (Lond.)* **301**:64–66 (1983).
9. Stephens, D. N., W. Kehr, H. H. Schneider, and R. Schmiechen. β-Carbolines with agonistic and inverse agonistic properties at benzodiazepine receptors of the rat. *Neurosci. Lett.* **47**:333–338 (1984).
10. Oakley, N. R., and B. J. Jones. Differential pharmacological effects of β-carboline-3-carboxylic acid esters. *Neuropharmacology* **21**:587–589 (1982).

11. Prado de Carvalho, L., P. Venault, M. C. Potier, R. H. Dodd, C. L. Brown, G. Chapouthier, and J. Rossier. 3-(Methoxycarbonyl)-amino-β-carboline, a selective antagonist of the sedative effects of benzodiazepine. *Eur. J. Pharmacol.* **129**:323–332 (1986).
12. Nielsen, M., and C. Braestrup. Ethyl-β-carboline-3-carboxylate shows differential benzodiazepine receptor interaction. *Nature* (*Lond.*) **286**:606–607 (1980).
13. Braestrup, C., and M. Nielsen. [^{3}H]Propyl β-carboline-3-carboxylate as a selective radioligand for the BZ_1 benzodiazepine receptor subclass. *J. Neurochem.* **37**:333–341 (1981).
14. Fehske, K. J., I. Zube, H. O. Borbe, U. Wollert, and W. E. Muller. β-Carboline binding indicates the presence of benzodiazepine receptor subclasses in the bovine central nervous system. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **319**:172–177 (1982).
15. Sieghart, W. Several new benzodiazepines selectively interact with a benzodiazepine receptor subtype. *Neurosci. Lett.* **38**:73–78 (1983).
16. Potier, M. C., L. Prado de Carvalho, R. H. Dodd, and J. Rossier. *In vivo* binding of [^{3}H]Ro 15-1788 in mice: comparison with the *in vivo* binding of [^{3}H]flunitrazepam. *Neuroscience* **22**:S98 (1987).
17. Dodd, R. H., C. Ouannes, L. Prado de Carvalho, A. Valin, P. Venault, G. Chapouthier, J. Rossier, and P. Potier. 3-Amino-β-carboline derivatives and the benzodiazepine receptor: synthesis of a selective antagonist of the sedative action of diazepam. *J. Med. Chem.* **28**:824–828 (1985).
18. Snedecor, G. W., and W. G. Cochran. *Statistical Methods.* Iowa State University Press, Ames, IA (1957).
19. Dubnick, B., A. S. Lippa, C. A. Klepner, J. Coupet, E. N. Greenblatt, and B. Beer. The separation of [^{3}H]benzodiazepine binding sites in brain and of benzodiazepine pharmacological properties. *Pharmacol. Biochem. Behav.* **18**:311–318 (1983).
20. Hirsch, J. D., and J. L. Lydigsen. Binding of β-carboline-3-carboxylic acid ethyl ester to mouse brain benzodiazepine receptors *in vivo*. *Eur. J. Pharmacol.* **72**:357–360 (1981).
21. Minchin, M. C. W., and D. J. Nutt. Studies on [^{3}H]diazepam and [^{3}H]ethyl-β-carboline carboxylate binding to rat brain *in vivo*: regional variations in displacement. *J. Neurochem.* **41**:1507–1512 (1983).
22. Lippa, A. S., D. Jackson, L. P. Wennogle, B. Beer, and L. R. Meyerson. Non-benzodiazepine agonists for benzodiazepine receptors, in *Pharmacology of Benzodiazepines.* (E. Udsin, P. Skolnick, J. F. Tallman, D. Greenblatt, and S. M. Paul, eds.). Macmillan Press, London, 431–440 (1982).
23. Paul, S. M., P. J. Syapin, B. A. Paugh, V. Moncada, and P. Skolnick. Correlation between benzodiazepine receptor occupation and anticonvulsant effects of diazepam. *Nature* (*Lond.*) **281**:688–689 (1979).
24. Duka, T., V. Hollt, and A. Herz. *In vivo* receptor occupation by benzodiazepines and correlation with the pharmacological effect. *Brain Res.* **179**:147–156 (1979).
25. Mennini, T., and S. Garattini. Benzodiazepine receptors: correlation with pharmacological responses in living animals. *Life Sci.* **31**: 2025–2035 (1982).
26. Braestrup, C., R. Schmiechen, G. Neef, M. Nielsen, and E. N. Petersen. Interaction of convulsive ligands with benzodiazepine receptors. Science (*Wash. D. C.*) **216**: 1241–1243 (1982).
27. Braestrup, C., and M. Nielsen. Benzodiazepine receptor binding *in vivo* and efficacy, in *Benzodiazepine/GABA Receptors and Chloride Channels: Structural and Functional Properties.* (R. W. Olsen and J. G. Venter, eds.), Vol. 5. Alan R. Liss, New York, 167–184 (1986).
28. Venault, P., G. Chapouthier, L. Prado de Carvalho, J. Simiand, M. Morre, R. H. Dodd, and J. Rossier. Benzodiazepine impairs and β-carboline enhances performance in learning and memory tasks. *Nature* (*Lond.*) **321**:864–866 (1986).
29. Grecksch, G., L. Prado de Carvalho, P. Venault, G. Chapouthier, and J. Rossier. Convulsions induced by submaximal dose of pentylenetetrazol in mice are antagonized by the benzodiazepine antagonist Ro 15-1788. *Life Sci.* **32**:2579–2584 (1983).
30. Perry, D. C., J. S. Rosenbaum, M. Kurowski, and W. Sadee. [^{3}H]Etorphine receptor binding *in vivo*: small fractional occupancy elicits analgesia. *Mol. Pharmacol.* **21**:272–279 (1982).
31. Goodman, L. S., and A. Gilman. *The Pharmacological Basis of Therapeutics.* Macmillan Press, New York, 22–23 (1967).
32. Bonetti, E. P., P. Lorenzo, R. Cumin, R. Schaffner, M. Pieri, E. R. Gamzu, R. K. M. Muller, and W. Haefely. Benzodiazepine antagonist Ro 15-1788: neurological and behavioral effects. *Psychopharmacology* **78**:8–18 (1982).

Send reprint requests to: M.-C. Potier, Laboratoire de Physiologie Nerveuse, CNRS, 91190 Gif-sur-Yvette, France.