bridge, Mass., 1965); Y. Katsuki, N. Suga, Y. Kanno, *J. Acoust. Soc. Am.* **34**, 1396 (1962); R. R. Pfeiffer and D. O. Kim, *ibid.* **52**, 1669 (1972).
6. M. C. Liberman, *J. Acoust. Soc. Am.* **63**, 442 (1976).
7. A. R. Light and E. G. Perl, *J. Comp. Neurol.* **186**, 133 (1979).
8. M. C. Liberman, *J. Acoust. Soc. Am.*, in press.
9. _____ and D. G. Beil, *Acta Oto-Laryngol.* **88**, 161 (1979).
10. J. C. Adams, *Neuroscience* **2**, 141 (1977).
11. H. H. Spoendlin, *Acta Oto-Laryngol.* **87**, 381 (1979).
12. R. J. Salvi, in *New Perspectives on Noise-Induced Hearing Loss* (Raven, New York, 1982); R. A. Schmiedt, paper presented at the midwinter research meeting of the Association for Research in Otolaryngology, St. Petersburg, Fla., 1982.
13. I thank L. W. Dodds, K. A. McKenney, and M. E. Oliver for their technical assistance and C. Gilbert for advice on intracellular injection techniques. This work was supported by PHS grants 2 P01 NS13126 and 5 P01 NS13126.

23 November 1981; revised 12 February 1982

Interaction of Convulsive Ligands with Benzodiazepine Receptors

Abstract. *The γ-aminobutyric acid (GABA)–benzodiazepine receptor complex, which is composed of distinct proteins embedded in the neuronal plasma membrane, is important for several effects of benzodiazepines, including protection afforded against convulsions. During structural modification of ethyl β-carboline-3-carboxylate an agent was discovered which has high affinity for brain benzodiazepine receptors but which is a potent convulsant. Also in contrast to benzodiazepines, this type of benzodiazepine receptor ligand favors benzodiazepine receptors in the non-GABA–stimulated conformation, which may explain the convulsive properties.*

Specific binding sites for benzodiazepines have been demonstrated in the central nervous system of higher vertebrates, including man (*1*). Evidence suggests that these binding sites are receptors for the benzodiazepine type of minor tranquilizers (*2*).

It was initially thought that only pharmacologically active benzodiazepines, and a few benzodiazepine-like agents, interacted with high affinity with these receptors (*2*). However, agents such as ethyl β-carboline-3-carboxylate (β-CCE) (*3*) and Ro 15-1788 (*4*) have been discovered which have a high affinity for benzodiazepine receptors but which lack the anticonvulsant and anticonflict effects of benzodiazepines (*4–6*). These agents can block or reverse benzodiazepines from eliciting their usual effects (*4–6*), indicating that their interaction with benzodiazepine receptors is functional and novel. In testing structural modifications of β-CCE we observed that methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) (Fig. 1) is a potent convulsant in mice and rats. Our findings suggest that DMCM produces convulsions by interacting with benzodiazepine receptors in a particular way. It appears that, by affecting the γ-aminobutyric acid (GABA)–benzodiazepine receptor complex (*7*), DMCM may reduce GABA-mediated neurotransmission.

To determine the nature of its convulsive properties we administered DMCM (1 to 300 mg/kg intraperitoneally) to mice (Fig. 1) or rats (5 to 10 mg/kg intraperitoneally). Clonic-tonic convulsions followed within 3 to 5 minutes. The convulsions were similar in quality to those produced by pentylenetetrazol; a short period of clonic fore- and hindlimb convulsions was followed by tonic extensor spasms. At DMCM doses above 15 mg/kg, all mice died within 10 to 15 minutes from respiratory depression. Less frequent and briefer convulsions were induced by methyl β-carboline-3-carboxylate (β-CCM) at 5 to 100 mg/kg intraperitoneally. The convulsive effects of DMCM and β-CCM occurred at doses at which these agents are bound to benzodiazepine receptors in living mice (Fig. 1). Similar effects were observed in rats (data not shown). As expected, convulsions induced by DMCM (15 mg/kg intraperitoneally) in mice were inhibited by benzodiazepines and barbiturates [mean effective dose (ED_{50}) for lorazepam, 0.5 mg/kg intraperitoneally; for diazepam, 9 mg/kg intraperitoneally; and for phenobarbital, 20 mg/kg orally]. The ability of Ro 15-1788 (ED_{50}, 7 mg/kg orally) and β-CCE (ED_{50}, 70 mg/kg, intraperitoneally, administered 35 minutes before DMCM) to inhibit convulsions induced by DMCM was surprising. Ro 15-1788 has been described as a pure benzodiazepine antagonist (*4*), but, to our knowledge, it has not been shown to antagonize convulsive agents. β-CCE not only fails to antagonize convulsions induced by pentylenetetrazol, picrotoxin, bicuculline, and strychnine, but actually has some proconvulsant activity—that is, it enhances the effect of several convulsive treatments (*5*). Both β-CCE and Ro 15-1788 can occupy benzodiazepine recep-

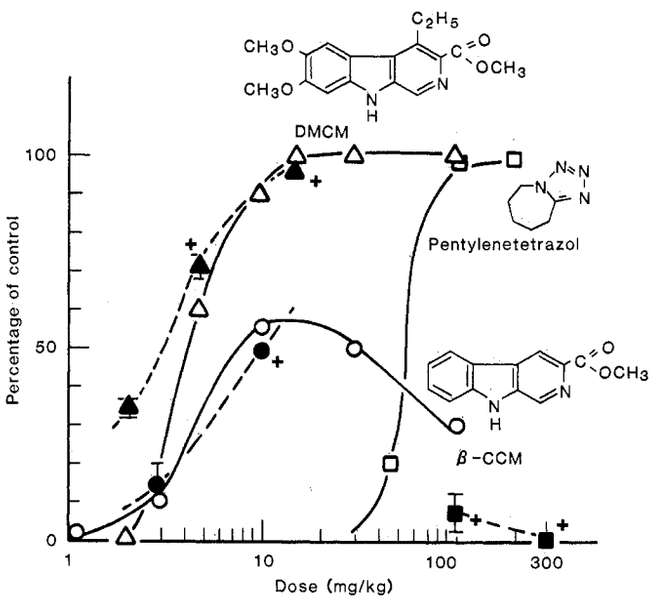

Fig. 1. Dose-related induction of clonic convulsions in male mice by DMCM (△), β-CCM (○) given intraperitoneally, and pentylenetetrazol (□) given subcutaneously. Relation to benzodiazepine receptor occupancy in vivo (filled symbols) as measured by inhibition of specific binding of [^{3}H]flunitrazepam mainly according to Chang and Snyder (*20*). DMCM and β-CCM were administered 15 minutes before decapitation, and pentylenetetrazol was administered 30 minutes before. Since binding of [^{3}H]flunitrazepam to receptors could not be determined in convulsing animals, we pretreated some animals (+) with phenobarbital (200 mg/kg orally, 45 minutes before decapitation). This dose of phenobarbital sodium did not by itself affect specific [^{3}H]flunitrazepam binding (data not shown). Vertical bars denote standard error of the mean (S.E.M.) values which extended outside the symbol.

0036-8075/82/0611-1241$01.00/0

tors, and the selective protection afforded by both agents against convulsions produced by DMCM suggests that benzodiazepine receptors are involved. The specificity of the interaction of DMCM with benzodiazepine receptors in vitro is supported by the observation that DMCM does not interact with a wide range of brain neurotransmitter receptors. For instance, no effect of DMCM ($10^{-5}M$) was observed on high-affinity binding sites for various ^{3}H-labeled ligands: lysergic acid diethylamide, spiroperidol, muscimol, WB 4101, naloxone, quinuclidinyl benzilate, and α-amino-3-hydroxy-5-methyl-4-isoxarole-propionic acid (AMPA) (data not shown). These ^{3}H-labeled ligands are presumed to reflect binding to serotonin, dopamine, GABA, noradrenaline, opiate, muscarine, and glutamate receptors, respectively.

To elucidate this seemingly specific mechanism of action of DMCM on benzodiazepine receptors, we considered the possibility that a particular class of benzodiazepine receptors was involved in the convulsive effects. There appear to be at least two types of receptor proteins or conformational classes of benzodiazepine receptors (*8*). The classes are designated BZ_1 and BZ_2 as characterized by their high and low affinity, respectively, for certain β-carboline esters (*9*). We used the ability of ligands to inhibit [^{3}H]flunitrazepam binding in cerebellum compared to that in hippocampus as a measure of BZ_1/BZ_2 selectivity (*9*). DMCM was slightly more potent in hippocampus [mean ± standard error of the mean (S.E.M.) inhibitory concentration (IC_{50}), 6.6 ± 1.3 n*M*, three experiments] than in cerebellum (IC_{50}, 10.9 ± 0.3 n*M*, three experiments), suggesting weak BZ_2 selectivity; β-CCM, a BZ_1 selective ligand, is four times more potent in cerebellum (*10*). Thus there is no clear relation between BZ_1/BZ_2 selectivity and pharmacological efficacy.

Another possible explanation for the effects of DMCM is that the ability of GABA and GABA agonists to enhance or reduce the affinity of ligands for benzodiazepine receptors reflects the pharmacological efficacy of the receptor ligands (*11*). These affinity changes, in turn, may reflect the ability of the ligands to enhance or reduce GABA-mediated chloride channel conductance. We therefore investigated the effect of GABA-receptor stimulation on the affinity for benzodiazepine receptors of DMCM and of other receptor ligands (Table 1). The ligands can be roughly divided into three overlapping groups according to whether muscimol enhances (GABA ratio > 1), leaves unaffected (GABA ratio ~ 1), or reduces (GABA ratio < 1) the affinity. Apparently the GABA ratio can be used to predict the pharmacological profile of benzodiazepine receptor ligands, since group 1 represents ligands with benzodiazepine-like pharmacology, group 2, ligands with little or no efficacy on benzodiazepine receptors, and group 3, ligands with effects opposite to those of benzodiazepines. It is not known whether the agents in group 3 represent convulsive or anxiogenic agents. For example, β-CCE and its methyl amide (FG 7142) are not convulsive when given alone to mice and rats. However, both agents can reverse anticonflict effects of phenobarbital, suggesting that they may be anxiogenic. Furthermore, in human volunteers FG 7142 is reported to produce intense fits of anxiety (*12*).

Table 1. Effect of GABA receptor stimulation on the affinity of ligands for benzodiazepine receptors in rat cerebellum. A GABA ratio of 2 means that GABA (or muscimol) enhances the affinity of the ligand for benzodiazepine receptors about twofold. Determinations were made as follows: specific binding of [^{3}H]diazepam (0.4 n*M*, 79 Ci/mmole, Amersham) was determined in an unfrozen, five times washed (tris-citrate, 100 m*M*, *p*H 7.1), rat cerebellar membrane preparation. Samples were incubated at 0°C for 20 minutes in a physiological buffer which was used for the final resuspension of membranes (NaCl, 122 m*M*; KCl, 4.8 m*M*; $CaCl_2$, 0.97 m*M*; $MgSO_4 \cdot 7H_2O$, 1.2 m*M*; and NaH_2PO_4, 16 m*M*; all final concentrations, *p*H 7.4). Four to six concentrations of the agents in duplicate were added to samples to determine the IC_{50} value in the presence and absence of a supramaximal concentration of muscimol ($10^{-5}M$), always in parallel experiments. A low concentration of [^{3}H]diazepam (about 1/10 of the dissociation constant value) was used to ensure that the experimentally determined IC_{50} values were close to the inhibition constant value. Values shown for the GABA ratio are means ± S.E.M. of the ratio of IC_{50} without muscimol to IC_{50} with muscimol added, as determined in independent experiments (*N*). Abbreviations: PrCC, propyl ester of β-CCE; CL 218872, 3-methyl-6-[3-(trifluoromethyl) phenyl]-1,2,4-triazolo[4,3-b]pyridazine; and Ro 15-1788, ethyl 8-fluro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate.

Ligand	GABA ratio	*N*
	Group 1	
Flunitrazepam	2.45 ± 0.2	4
Estazolam	2.43	2
Oxazepam	2.35	2
Diazepam	2.30 ± 0.3	4
Chlordiazepoxide	2.23 ± 0.1	3
Clonazepam	2.12 ± 0.2	3
CL 218872	1.98 ± 0.1	4
Lorazepam	1.75	2
Lormetazepam	1.71	2
Zopiclone	1.53 ± 0.1	3
	Group 2	
Ro 15-1788	1.22 ± 0.1	3
PrCC	1.11	2
	Group 3	
β-CCE	0.86 ± 0.1	4
FG 7142	0.87 ± 0.1	4
β-CCM	0.61	2
DMCM	0.46 ± 0.1	3

To illustrate how benzodiazepine receptor ligands might affect GABA-mediated transmission, suppose that there are two conformations of the receptors which are in equilibrium. Opening of neuronal chloride channels by GABA is dependent on the benzodiazepine receptor being in a particular activated conformation (open chloride channel form). Benzodiazepines with high affinity for the activated form might shift the equilibrium and stabilize this conformation, which would increase the probability that a chloride channel would be opened by GABA receptor occupation (*13*). Benzodiazepines alone rarely cause chloride channel opening (*13*). However, DMCM, which may have high affinity for the closed chloride channel conformation (Table 1, GABA ratio < 1), would tend to lock the receptors in a conformation that reduces the probability of opening chloride channels, and would thus reduce GABA-mediated neurotransmission. This possibility is supported by the observation that DMCM reduces electrophysiological effects of GABA in cultures of spinal neurons (*14*). True competitive benzodiazepine receptor antagonists would not favor either conformation and would not affect GABA-mediated transmission. These antagonists, however, would obstruct other benzodiazepine receptor ligands and thereby antagonize agents that act through these receptors.

DMCM is convulsant through its action on the benzodiazepine recognition site in the GABA-benzodiazepine receptor complex. Ro 5-3663, which is chemically related to benzodiazepines but has a negligible affinity for benzodiazepine receptors, is convulsant probably through an interaction with chloride channels which is similar, but not identical, to that of the convulsant barbiturates and picrotoxin (*15*). The mechanism of action of pentylenetetrazol is probably also related to chloride channels (*16*) and not to an interaction with the recognition sites of benzodiazepine receptors (Fig. 1). Bicuculline produces convulsions by a competitive interaction at the GABA receptor recognition site; strychnine antagonizes glycine in the spinal cord (*17*). Some harmal alkaloids of β-carboline structure produce tremor or weak convulsions, but their interaction with benzodiazepine receptors in living animals has not been demonstrated (*18*).

Our results show that benzodiazepine receptor ligands may interact with the receptors in different ways, producing a spectrum of pharmacological effects. Some barbiturates may represent a similar continuum of receptor ligands interacting with their target in different ways (*19*).

C. BRAESTRUP
A/S Ferrosan, Research Laboratory, 5 Sydmarken, DK-2860 Soeborg, Denmark, and *Psychopharmacological Research Laboratory, St. Hans Hospital, DK-4000 Roskilde, Denmark*
R. SCHMIECHEN
G. NEEF
Schering AG, West Berlin, Federal Republic of Germany
M. NIELSEN
Psychopharmacological Research Laboratory, St. Hans Hospital
E. N. PETERSEN
A/S Ferrosan, Research Laboratory

References and Notes

1. R. F. Squires and C. Braestrup, *Nature (London)* **266**, 732 (1977); C. Braestrup and R. F. Squires, *Br. J. Psychiatry* **133**, 249 (1978); H. Möhler and T. Okada, *Life Sci.* **20**, 2101 (1977).
2. J. F. Tallman, S. M. Paul, P. Skolnick, D. W. Gallager, *Science* **207**, 274 (1980); C. Braestrup and M. Nielsen, *Trends Pharmacol. Sci.* **1**, 424 (1980).
3. C. Braestrup, M. Nielsen, C. E. Olsen, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2288 (1980).
4. W. Hunkeler *et al.*, *Nature (London)* **290**, 514 (1981).
5. S. S. Tenen and J. D. Hirsch, *ibid.* **288**, 609 (1980); P. J. Cowen, A. R. Green, D. J. Nutt, *ibid.* **290**, 54 (1981); C. Cepeda, T. Tanaka, R. Besselièvre, P. Potier, R. Naquet, J. Rossier, *Neurosci. Lett.* **24**, 53 (1981).
6. R. Mitchell and I. Martin, *Eur. J. Pharmacol.* **68**, 513 (1980); R. A. O'Brien *et al.*, *Life Sci.* **29**, 75 (1981); P. Polc, N. Ropert, D. M. Wright, *Brain Res.* **217**, 216 (1981); J. D. Hirsch and J. L. Lydigsen, *Eur. J. Pharmacol.* **72**, 357 (1981).
7. J. F. Tallman, J. W. Thomas, D. W. Gallager, *Nature (London)* **274**, 383 (1978).
8. R. F. Squires *et al.*, *Pharmacol. Biochem. Behav.* **10**, 825 (1979); see also review by C. Braestrup and M. Nielsen [*Trends Neurosci.* **1980**, 301 (December 1980).
9. C. Braestrup and M. Nielsen, *J. Neurochem.* **37**, 333 (1981).
10. See C. Braestrup, M. Nielsen, H. Skovbjerg, and O. Gredal [in *GABA and Benzodiazepine Receptors*, E. Costa, G. DiChiara, G. L. Gessa, Eds. (Raven, New York, 1981), p. 147] for methodological details and values for comparison.
11. F. J. Ehlert, W. R. Roeske, C. Braestrup, S. H. Yamamura, H. I. Yamamura, *Eur. J. Pharmacol.* **70**, 593 (1981); C. Braestrup and M. Nielsen, *Nature (London)* **294**, 472 (1981).
12. R. Dorow, unpublished observations.
13. D. W. Gallager, *Eur. J. Pharmacol.* **49**, 133 (1978); D. W. Choi, D. H. Farb, G. D. Fischbach, *J. Neurophysiol.* **45**, 621 (1981); R. E. Study and J. L. Barker, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7180 (1981).
14. J. Lambert, unpublished observations.
15. W. Schlosser and S. Franco, *J. Pharmacol. Exp. Ther.* **211**, 290 (1979); R. A. O'Brien and N. M. Spirt, *Life Sci.* **26**, 1441 (1980); R. W. Olsen, *J. Neurochem.* **37**, 1 (1981).
16. R. L. MacDonald and J. L. Barker, *Neurology* **28**, 325 (1978).
17. D. R. Curtis and G. A. R. Johnston, in *Neuropoisons*, L. L. Simpson, Ed. (Plenum, New York, 1974), p. 207.
18. H. A. Robertson, *Eur. J. Pharmacol.* **67**, 129 (1980); A. M. Morin, I. A. Tanaka, C. G. Wasterlain, *Life Sci.* **28**, 2257 (1981); H. Rommelspacher, C. Nanz, H. O. Borbe, K. J. Fehske, W. E. Müller, U. Wollert, *Eur. J. Pharmacol.* **70**, 409 (1981).
19. F. Leeb-Lundberg and R. W. Olsen, *Mol. Pharmacol.*, in press.
20. R. Chang and S. H. Snyder, *Eur. J. Pharmacol.* **48**, 213 (1978).

21 January 1982; revised 10 March 1982

Flexor Reflex Control of the External Sphincter of the Urethra in Paraplegia

Abstract. *In paraplegics and quadriplegics a profound paralysis of skeletal muscles occurs below the level of the spinal lesion. Unexplained in this state is the development of an overactive external urethral sphincter, which interferes with emptying of the bladder and may lead to infection of the urinary tract. Studies of cats show that the discharge of motoneurons causing this contraction has all the characteristics of a flexor reflex.*

In paraplegia and quadriplegia of an upper motoneuron type, overactivity of the external urethral sphincter (EUS) develops, preventing the passage of urine. The discharge of the motoneurons responsible for this overactivity has been poorly understood. It has long been known that certain natural or experimental stimuli below the level of the spinal lesion affect the activity of the EUS. The reflex pattern of these effects and their functional significance in controlling the EUS, however, have never been adequately analyzed. In experiments on rats and cats, we noted that stimulation of cutaneous nerves in the hind limb elicits responses in ipsilateral nerves to the EUS similar to the discharges in flexor muscle nerves. Lloyd (*1*) classified the latter as flexor reflexes.

We report that these hitherto unclassified responses of the EUS and the efferent discharges in its nerves meet the accepted criteria for flexor reflexes in limb muscles. Local anesthesia of the skin and subcutaneous tissues of the hind limbs abolishes these flexor reflexes and the tonic efferent activity in the nerves to the EUS, suggesting that sensory inputs from these areas are essential elements in the overactivity of the EUS.

In experiments on cats whose spinal cords were transected at the obex (*2*), the same types of stimuli that elicited flexor reflexes in limb muscles evoked contractions in sphincter muscles. Nociceptive stimuli that evoked strong contractions in ipsilateral flexor muscles also elicited brisk contractions of the EUS. If one paw was pricked with a needle, for example, there was a sudden withdrawal of that limb due to contractions of flexor muscles in the hip, thigh, and leg. The same stimulus caused the EUS and anal sphincter to contract. Non-nociceptive stimuli—such as moderate heat, cold, pressure, and touch—applied to the skin of the thighs, legs, and feet evoked similar but less vigorous contractions in flexor muscles of the ipsilateral limb and also caused contractions of the urethral and anal sphincters. Electrical stimulation of these regions elicited almost simultaneous contractions of ipsilateral flexor muscles and both sphincters.

The distribution of reflex responses is one of the most important criteria in classifying them. Single-shock stimulation of cutaneous nerves (sural or superficial peroneal) in cats with high spinal lesions usually results in reflex contractions of flexor muscles in the ipsilateral hind limb. No contractions occur in ipsilateral extensor muscles, and responses evoked in these muscles by appropriately timed stimuli are generally inhibited during flexor reflexes. Contralaterally, the extensor muscles are excited and the flexors are inhibited.

Figure 1A shows three simultaneously recorded responses to stimulation of the left common peroneal nerve (a mixed cutaneous and muscle nerve). Trace a illustrates the absence of any reflex response in the ipsilateral gastrocnemius nerve (extensor); trace b illustrates a typical polysynaptic flexor discharge recorded from the ipsilateral biceps femoris nerve (flexor); and trace c is the response recorded from the nerve to the EUS. The reflex in the nerve to the EUS is part of a widespread response in a group of other pelvic floor and limb flexor muscles. These examples illustrate some of the similarities in the distribution of reflex responses in the nerves to flexor muscles and to the urethral and anal sphincters (*3*).

Another criterion in the classification of flexor reflexes is that they can be inhibited by (i) prior stimulation of nerves to direct antagonists or of some other extensor nerves and by (ii) elicitation of other types of ipsilateral extensor reflexes (*4*). Trace a in Fig. 1B illustrates the response of the nerve to the EUS to stimulation of the common peroneal nerve. Most of this large reflex was inhibited by delivering a single shock to the gastrocnemius nerve 10 msec before the test shock (trace b in Fig. 1B). The

0036-8075/82/0611-1243S01.00/0