Pharmacology Biochemistry & Behavior, Vol. 23, pp. 681–685, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Heterogeneity of Benzodiazepine Binding Sites: A Review of Recent Research

JAMES D. HIRSCH, KENNON M. GARRETT AND BERNARD BEER[1]

Department of Central Nervous System Research, Medical Research Division, American Cyanamid Company Lederle Laboratories, Pearl River, NY 10965

HIRSCH, J. D., K. M. GARRETT AND B. BEER. *Heterogeneity of benzodiazepine binding sites: A review of recent research.* PHARMACOL BIOCHEM BEHAV **23**(4) 681–685, 1985.—This paper reviews selected aspects of benzodiazepine binding site heterogeneity. These include receptor heterogeneity revealed by biochemical determinations of receptor numbers, autoradiographic localization in histological sections of brain, lesion studies, solubilization of receptors, and photoaffinity labelling. The data summarized support the concept of benzodiazepine receptor multiplicity. In addition, we have reviewed recent work on peripheral-type benzodiazepine binding sites and suggest that further study of these sites may increase our understanding of both the central and peripheral actions of benzodiazepines and other ligands.

Benzodiazepines Binding sites Heterogeneity Lesions Autoradiography Photoaffinity labeling

THE development of an anxiolytic without sedative or muscle relaxant properties has been a major goal in pharmaceutical research. The currently marketed anxiolytics all share very similar pharmacological effects in animals and man. Aside from their anti-anxiety and anti-convulsive properties, they also produce sedation, ataxia and decreased muscle tone. These motoric effects are significantly potentiated by alcohol. Because of this pharmacological commonality, it was assumed by many that these side effects were induced by the same physiological mechanism that generated the clinical efficacy in treating anxiety. It was, in fact, accepted that the sedative properties were a component of anxiolytic activity.

Subsequent research showed that anxiolytic properties were separable from undesirable side effects. In the early 1970's it was demonstrated that there was a significant correlation between a compound's anti-anxiety (anti-punishment) effects and its ability to inhibit cyclic AMP phosphodiesterase [3]. Whether cyclic nucleotides contribute to the pharmacological properties of anxiolytics is still not clearly understood. However, this research did provide the first series of non-sedating anxiolytic drugs, the pyrazolopyridines [3]. The triazolopyridazine, CL 218,872, was first described in 1979 and it has become the focus of considerable research. Like the benzodiazepines, CL 218,872 and the other triazolopyridazines increased punished responding in a conflict paradigm and protected against convulsions produced by pentylenetetrazole. These pharmacological properties are highly predictive of anxiolytic activity. However, unlike the benzodiazepines, CL 218,872 was relatively inactive in tests designed to measure effects on neuronal systems which utilize GABA or glycine as transmitters. Furthermore, CL 218,872 was relatively free of the ataxic and depressant side effects commonly associated with the benzodiazepines and was considerably less interactive with alcohol [17, 19, 43].

The *in vitro* interactions of CL 218,872 with brain benzodiazepine receptors are also novel and significant. Initial reports indicated that brain benzodiazepine receptors were a single, homogeneous class of sites which mediated the major actions of benzodiazepines [22, 30, 43]. As often occurs in receptor studies, further work demonstrated apparent heterogeneity of binding sites. The key observation which first suggested the possibility of multiple benzodiazepine receptors and multiple substrates for anxiolytic action was that CL 218,872 not only bound with high affinity to brain receptors, but selectively interacted with a subclass of receptors designated as Type I or BZ_1 [17]. CL 218,872 and the other triazolopyridazines have higher affinity for Type I receptors which suggests that these sites play a role in the anxiolytic and anticonvulsant effects of these drugs. The benzodiazepines have equal affinity for Type I receptors and the other subclass, Type II or BZ_2, which, by analogy, may be primarily responsible for sedation and muscle relaxation. The existence of nonbenzodiazepine drugs with biological and biochemical properties like CL 218,872 stimulated discovery research on new chemical classes of potential anxiolytics and created intense interest in the multiplicity of brain benzodiazepine receptors. Anxioselective anxiolytics were recently reviewed in detail [47]. The present review will highlight selected aspects of benzodiazepine receptor heterogeneity.

RECEPTOR HETEROGENEITY REVEALED BY RECEPTOR NUMBER DETERMINATION

In addition to CL 218,872, the β-carbolines have been used effectively to demonstrate benzodiazepine receptor heterogeneity *in vitro*. Searching for endogenous ligands for

[1]Requests for reprints should be addressed to B. Beer.

TABLE 1

β-CARBOLINE AND BENZODIAZEPINE LIGANDS LABEL UNEQUAL NUMBERS OF RECEPTORS IN MAMMALIAN BRAIN REGIONS

Region	B_{max} (fmol/mg protein) ^{3}H-BDZ	^{3}H-PCC	PCC/BDZ	Reference
cerebellum	730	580	0.79	[14]
cortex	1207	860	0.71	[8]
retina	692	437	0.63	[11]
pineal*	56	34	0.60	[21]
hippocampus	939	533	0.57	[8]
hypothalamus	1025	265	0.26	[13]

*Data in pineal was obtained with ^{3}H-ECC as the ligand.

the receptor, Braestrup and coworkers isolated ethyl β-carboline-3-carboxylate (ECC) from human urine [9]. This compound was later shown not to be an endogenous ligand, but it bound with high affinity to the receptor *in vitro* [9,45] and occupied the receptor *in vivo* [15]. Ethyl β-carboline-3-carboxylate was also the first compound shown to antagonize the actions of benzodiazepines through binding to the receptor [45]. Binding studies with ^{3}H-propyl β-carboline-3-carboxylate (PCC), an analog of ECC, have consistently revealed that this ligand labels fewer total receptors (B_{max}) in mammalian brain membranes than benzodiazepines (Table 1). For example, PCC labels about 80% of the sites labeled by benzodiazepines in cerebellum. In contrast, only 25% of the benzodiazepine-labeled sites in the hypothalamus are occupied by PCC, while B_{max} values in the hippocampus and other regions fall between these two extremes.

Although the arguments about whether β-carbolines and benzodiazepines occupy physically separate receptors or non-equivalent domains on individual receptor molecules *in vitro* have not been settled, unequal numbers of binding sites for these types of ligands clearly support some kind of receptor heterogeneity.

Another approach to exploring the meaning of unequal B_{max} values in different brain regions would be to compare B_{max} values for Type I-specific ^{3}H-CL 218,872 with those obtained with ^{3}H-benzodiazepines. Unfortunately, there are only a few *in vitro* binding studies with ^{3}H-CL 218,872 [33,48] and technical difficulties with this ligand have prevented full exploitation of its Type I receptor specificity in direct binding experiments. However, if we accept the claims that ^{3}H-PCC is also a specific ligand for Type I receptors [8], then it can be suggested that unequal numbers of β-carboline binding sites in different brain regions represent different quantities of Type I receptors in these regions.

RECEPTOR HETEROGENEITY REVEALED BY *IN SITU* AUTORADIOGRAPHIC LOCALIZATION IN BRAIN SECTIONS

Triazolopyridazines like CL 218,872 have been instrumental in delineating the existence of Type I and II receptors in brain membrane homogenates. However, the ligand recognition specificity of receptors in extensively disrupted neural tissue might be different from that shown in intact brain. Recent studies of the autoradiographic localization of benzodiazepine receptors in histological sections of brain have confirmed the Type I-Type II concept. Selective displacement of ^{3}H-flunitrazepam (^{3}H-FLU) by CL 218,872 was observed in slices of rat brain regions, like cerebellum, which have been shown in homogenate studies to contain predominantly Type I receptors [50]. Type II receptors have been autoradiographically localized to the hippocampus, superior colliculus, and caudate-putamen [50]. In human amygdala, the distribution of Type I and II receptors was mapped by CL 218,872 displacement of ^{3}H-FLU and was different from that observed in rat amygdala [34]. Similar phylogenetic differences were observed in cerebellum [49]. More recently, Benevides and coworkers have confirmed the differential localization of Type I and II receptors in rat brain sections [4], and showed that the specificity of CL 218,872 was still observed at an elevated incubation temperature.

RECEPTOR HETEROGENEITY REVEALED BY LESION STUDIES, RECEPTOR SOLUBILIZATION AND PHOTOAFFINITY LABELING

Along with the autoradiographic evidence that the Type I and Type II BDZ receptors are differentially distributed among brain regions, recent biochemical studies have shown that there are differences in the morphologic distribution of benzodiazepine receptor subtypes within brain membranes. Lo and his colleagues [20] have demonstrated that Type I and Type II BDZ receptors are differentially solubilized with Triton X-100. Equilibrium binding of ^{3}H-FLU and ^{3}H-PCC to Triton X-100-soluble and insoluble fractions revealed that Type II receptors were solubilized by the detergent, while Type I receptors remained in the particulate fraction. This suggests that there is a physicochemical difference between the subtypes and/or a difference in the membrane matrix which surrounds the receptors. Lesioning brain noradrenergic pathways with 6-OHDA [26] produced a decrease in the number of ^{3}H-FLU binding sites, but did not alter the binding of ^{3}H-ECC. Also, studies using ibotenic acid to selectively lesion components of the striatonigral pathway showed that destruction of the afferent pathways caused a decrease in the number of Type II receptors and a proliferation of Type I receptors. Destruction of cell bodies in the *substantia nigra* decreased the number of Type I receptors, but had no effect on the number of Type II receptors [20]. These data suggest that the Type I receptors are located primarily on postsynaptic membranes while Type II receptors reside on presynaptic nerve terminals.

The concept of multiple benzodiazepine receptors has been further supported by experiments of Sieghart and his colleagues in which they photoaffinity labeled benzodiazepine receptors with ^{3}H-FLU [38, 40, 41]. Benzodiazepine receptors are irreversibly labeled with ^{3}H-FLU when the incubation mixture is irradiated with UV light [29]. Separation of the photoaffinity-labeled proteins by SDS-PAGE revealed two major labeled protein bands with molecular weights of 51000 (P51) and 55000 (P55) daltons. In addition, the proportions of these two bands varied among brain regions. In the hippocampus, hypothalamus, striatum and retina the P51 protein comprised 50–60% of the total photoaffinity-labeled protein while the P55 protein made up 15–20% of the irreversibly labeled protein. In contrast, the proportion of P51 in the inferior colliculus, cerebellum and substantia nigra was about 80–90% while the P55 protein comprised only 3–6% of the total labeled protein [38].

Photoaffinity incorporation of ^{3}H-FLU into the P51 and P55 proteins was also differentially affected by benzodiazepine receptor ligands. Sieghart and coworkers [41] showed that CL 218,872 and ECC were ten times more po-

tent in inhibiting photoaffinity labeling of the P51 band than the P55 band in the hippocampus. The IC_{50} values for the inhibition by CL 218,872 and ECC of photo-incorporation of ^{3}H-FLU into the P51 band in the cerebellum were similar to the IC_{50} values obtained from the P51 protein in the hippocampus.

These data, when compared to the results from inhibition of equilibrium ^{3}H-FLU binding in membrane preparations by CL 218,872 and ECC, suggested that the P51 protein corresponded to the Type I BDZ receptor while the P55 protein represented the Type II BDZ receptor. The regional distribution of the Type I and Type II BDZ receptors was similar to the distribution of the P51 and P55 proteins. Type I and Type II BDZ receptors had equal affinity for reversible ^{3}H-FLU binding and the P51 and P55 proteins had equal affinity for photoaffinity labeling by ^{3}H-FLU. In addition, the affinities of the Type I and Type II BDZ receptors for reversible ^{3}H-FLU binding were almost identical to the affinities for ^{3}H-FLU photoaffinity labeling [40]. Finally, the affinities of CL 218,872 and ECC for the Type I receptor were very similar to the affinities of the ligands for the P51 protein. The affinities of the two drugs for the Type II receptor matched their affinites for the P55 protein.

Through the use of increasingly more discriminating techniques, the concept of biochemically heterogeneous benzodiazepine receptors has become well established. However, none of these techniques alone has been sufficient to prove that Type I and II receptors subserve the anxiolytic and other actions of benzodiazepines, respectively. The extremely diverse pharmacological effects of many ligands for benzodiazepine receptors suggest that there may be more to functional heterogeneity of receptors than can be explained *solely* by the Type I-Type II concept. Strategies for further research based upon the heterogeneous receptor concept may provide answers to critical, clinically relevant questions about the functions of these receptors in the CNS.

RECEPTOR HETEROGENEITY REVEALED BY STUDYING NON-NEURONAL CELLS

In their first paper describing brain benzodiazepine receptors, Squires and Braestrup reported the presence of apparent specific, high affinity ^{3}H-diazepam binding in several peripheral tissues [10]. In contrast to the brain receptor, the non-anxiolytic but centrally active benzodiazepine, Ro5-4864 [51], inhibited peripheral-type binding potently. A number of studies since 1977 using ^{3}H-benzodiazepines, ^{3}H-Ro5-4864, and other peripheral-site-specific ligands [5] have demonstrated that distinct binding sites for benzodiazepines exist in a wide variety of peripheral tissues. Such sites are also present on the olfactory nerves, glial cells, ependymal cells, and on the choroid plexus [1] in the CNS. These sites have high affinity for Ro5-4864 and other Ro derivatives, PK11195 [18], and diazepam, lower affinity for flunitrazepam and virtually no affinity for clonazepam, Ro15-1788 and Cl 218,872 [1]. They appear to be primarily intracellular rather than on the outer membrane, and several reports indicate their association with mitochondria [12,23]. A nuclear association of putative peripheral-type sites has even been reported [7]. Peripheral-type sites are unaffected by GABA and other potent effectors of central-type receptors [35] and are not inactivated by photoaffinity labeling [23]. Peripheral-type sites from kidney have also been solubilized and partially purified [24].

Until recently, no functional role in the CNS or elsewhere could be suggested for peripheral-type sites and some investigators have called them irrelevant "drug acceptors" [37]. However, an evaluation of recent research suggests that peripheral-type sites are tied to cellular function in several ways. Wang and coworkers have reported a strong positive correlation between IC_{50} values for ^{3}H-Ro5-4864 binding and the corresponding EC_{50} values for inhibiting ^{3}H-thymidine incorporation in cultured thymoma cells [46]. Other work in culture demonstrated that benzodiazepines which were most potent at binding to peripheral-type sites (Ro5-4864, diazepam, flunitrazepam) were also the most potent at inducing melanogenesis in B16/C3 melanoma cells [25]. Although ligand binding data were not provided, Nagele *et al.* have reported that increasing concentrations of diazepam inhibited the division and spreading of chick embryo fibroblasts and produced a decrease in myosin content in these cells [32]. These effects of diazepam are consistent with the antiproliferative effects of this drug on thymoma cells [46]. We and others [31] have identified peripheral-type sites on lymphocytes, which may be tied to anti-proliferative and anticytotoxic effects of Ro5-4864 on these cells in culture (J. D. Hirsch and C. A. Mullen, unpublished observations). Very recently, in a series of studies in guinea pig heart, Mestre *et al.* [27,28] have demonstrated that peripheral site ligands significantly decrease contractility via peripheral site coupling to putative calcium channels.

These data suggest that it is ill-advised to discard peripheral-type benzodiazepine binding sites as irrelevant and non-functional drug acceptors, and we agree with Williams who suggested that benzodiazepines and anxiety should no longer be considered as inseparable entities [47]. Drugs that bind to peripheral-type sites do have pharmacologically-defined activities in a number of systems, including the brain, and our knowledge of Ro5-4864 pharmacology is evolving rapidly [36]. Clearly, Ro5-4864 is not an anxiolytic [36,51]; it is, however, centrally and peripherally active. It should also be recognized that the affinity of diazepam for peripheral-type sites in the brain is not much lower than its affinity for central-type receptors. Thus, peripheral-type sites may even play a role in the well-recognized CNS effects of this drug. We suggest that pursuit of the pharmacology and physiology of peripheral sites with the same intensity as central-type receptors may well open up new therapeutic areas in the future.

CONCLUSIONS

In this paper, we have reviewed selected recent findings about the biochemical heterogeneity of central benzodiazepine receptors. Great progress has been made in characterizing these recognition sites and delineating their molecular pharmacology. However, the difficult task of understanding how central receptor subtypes mediate the multiple actions of benzodiazepine and other receptor ligands remains. Recent studies of *in situ* receptor dynamics in cultured neurons [6] and the induction of functional, membrane-incorporated receptors by injection of brain messenger RNA into frog oocytes [16], may provide new biochemical and physiological approaches to understanding receptor function. Moreover, new drugs with selectivity for central receptor subtypes are now available for crucial *in vivo* studies of the functional heterogeneity of receptors [38, 42, 47].

We have also included a brief discussion of peripheral-type benzodiazepine binding sites. Our appreciation of their potential significance is growing as ligands for studying their molecular pharmacology and physiological role become

available. It may also be possible to study the biological functions of peripheral-types sites in cell culture where binding site occupancy and its after-effects are more readily accessible. Pursuit of endogenous ligands for peripheral-type sites [2] may be successful and allow us to investigate the role(s) of these sites *in vivo* as well.

ACKNOWLEDGEMENTS

The authors would like to thank Carmela Nardella and Carol Mullen for excellent secretarial and editoral assistance.

REFERENCES

1. Anholt, R. R. H., K. M. M. Murphy, G. E. Mack and S. H. Snyder. Peripheral-type benzodiazepine receptors in the central nervous system: localization to olfactory nerves. *J Neurosci* **4:** 593–603, 1984.
2. Beaumont, K., A. K. Cheung, M. C. Geller and D. D. Fanestil. Inhibitors of peripheral-type benzodiazepine receptors present in human urine and plasma ultrafiltrates. *Life Sci* **33:** 1375–1384, 1983.
3. Beer, B., M. Chasin, D. E. Clody, J. R. Vogel and Z. P. Horovitz. Cyclic adenosine monophosphate phosphodiesterase in brain: effect on anxiety. *Science* **176:** 428–430, 1972.
4. Benavides, J., C. Malgouris, A. Flamier, C. Tur, D. Quartonet, F. Begassot, J. C. Camelin, A. Uzan, C. Gueremy and G. LeFur. Biochemical evidence that 2-phenyl-4 [3-(4-piperidinyl) ethyl] quinoline, a quinoline derivative with pure anticonflict properties, is a partial agonist of benzodiazepine receptors. *Biochem Pharmacol* **23:** 1129–1136, 1984.
5. Benavides, J., C. Malgouris, F. Imbault, F. Begassat, H. Uzan, C. Renault, M. C. Dubroeucq, C. Gueremy and G. LeFur. "Peripheral Type" benzodiazepine binding sites in rat adrenals: binding studies with ^{3}H-PK 11195 and autoradiographic localization. *Arch Int Pharmacodyn Ther* **266:** 38–49, 1983.
6. Borden, L. A., C. Czajkowski, C. Y. Chan and D. H. Farb. Benzodiazepine receptor synthesis and degradation by neurons in culture. *Science* **226:** 857–860, 1984.
7. Bosmann, H. B., D. P. Penney, K. R. Case and K. Averill. Diazepam receptor: specific nuclear binding of ^{3}H-flunitrazepam. *Proc Natl Acad Sci USA* **77:** 1195–1198, 1980.
8. 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.
9. 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.
10. 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.
11. 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 Schmiedebergs Arch Pharmacol* **319:** 172–177, 1982.
12. Hirsch, J. D. Pharmacological and physiological properties of benzodiazepine binding sites in rodent brown adipose tissue. *Comp Biochem Physiol (C)* **77:** 339–343, 1984.
13. Hirsch, J. D. and R. L. Kochman. Occupation of brain receptors by benzodiazepines and β-carbolines: multiple mechanisms and responses. *Drug Dev Res* **4:** 39–50, 1984.
14. Hirsch, J. D., R. L. Kochman and P. R. Sumner. Heterogeneity of brain benzodiazepine receptors demonstrated by ^{3}H-propyl β-carboline-3-carboxylate binding. *Mol Pharmacol* **21:** 618–628, 1982.
15. 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.
16. Houamed, K. M., G. Bilbe, T. G. Smart, A. Constanti, D. A. Braun, E. A. Barnard and B. M. Richards. Expression of functional GABA, glycine and glutamate receptors in *Xenopus* oocytes injected with rat brain mRNA. *Nature* **310:** 318–321, 1984.
17. Klepner, C. A., A. S. Lippa, D. I. Benson, M. C. Sano and B. Beer. Resolution of two biochemically and pharmacologically distinct benzodiazepine receptors. *Pharmacol Biochem Behav* **11:** 457–462, 1979.
18. LeFur, G., M. L. Perrier, N. Vaucher, F. Imbault, A. Flamier, J. Benavides, A. Uzan, C. Renault, M. C. Dubroeucq and C. Gueremy. Peripheral benzodiazepine binding sites: effects of PK 11195, 1-(2-chlorophenyl)-N-methyl-N-(1-methyl propyl)-3-isoquinoline carboxamide. I. *in vitro* studies. *Life Sci* **32:** 1839–1847, 1983.
19. Lippa, A. S., J. Coupet, E. N. Greenblatt, C. A. Klepner and B. Beer. A synthetic non-benzodiazepine ligand for benzodiazepine receptors: a probe for investigating neuronal substrates of anxiety. *Pharmacol Biochem Behav* **11:** 99–106, 1979.
20. Lo, M. M. S., D. L. Niehoff, M. J. Kuhar and S. H. Snyder. Differential localization of type I and type II benzodiazepine binding sites in substantia nigra. *Nature* **306:** 57–60, 1983.
21. Lowenstein, P. R. and D. P. Cardinale. Characterization of flunitrazepam and beta-carboline high affinity binding in bovine pineal gland. *Neuroendocrinology* **37:** 150–154, 1983.
22. Mackerer, C. R., R. L. Kochman, B. A. Bierschenk and S. S. Bremner. The binding of ^{3}H-diazepam to rat brain homogenates. *J Pharmacol Exp Ther* **206:** 405–413, 1978.
23. Marangos, P. J., J. Patel, P. Boulenger and R. Clark-Rosenberg. Characterization of peripheral-type benzodiazepine binding sites in brain using ^{3}H-Ro5-4864. *Mol Pharmacol* **22:** 26–32, 1982.
24. Martini, C., G. Giannaccini and A. Lucacchini. Solubilization of rat kidney benzodiazepine binding sites. *Biochem Biophys Acta* **728:** 289–292, 1983.
25. Matthew, E., J. D. Laskin, E. Zimmerman, I. B. Weinstein, K. C. Hsu and D. L. Engelhardt. Benzodiazepines have high-affinity binding sites and induce melanogenesis in B16/C3 melanoma cells. *Proc Natl Acad Sci USA* **78:** 3939–3939, 1981.
26. Medina, J. H., M. L. Novas and E. DeRobertis. Heterogeneity of benzodiazepine receptors: Experimental differences between (^{3}H)Flunitrazepam and (^{3}H)Ethyl-β-Carboline-3-carboxylate binding sites in rat brain membranes. *J Neurochem* **41:** 703–709, 1983.
27. Mestre, M., T. Carriot, C. Belin, A. Uzan, C. Renault, M. C. Dubroeucq, C. Gueremy, A. Doble and G. LeFur. Electrophysiological and pharmacological evidence that peripheral type benzodiazepine receptors are coupled to calcium channels in the heart. *Life Sci* **36:** 391–400, 1985.
28. Mestre, M., T. Carriot, C. Belin, A. Uzan, C. Renault, M. D. Dubroeucq, C. Gueremy and G. LeFur. Electrophysiological and pharmacological characterization of peripheral benzodiazepine receptors in guinea pig heart preparation. *Life Sci* **35:** 953–962, 1984.
29. Mohler, H., M. K. Battersby and J. G. Richards. Benzodiazepine receptor protein identified and visualized in brain tissue by a photoaffinity label. *Proc Natl Acad Sci USA* **77:** 166–167, 1980.

30. Mohler, H. and T. Okada. Benzodiazepine receptor: demonstration in the central nervous system. *Science* **198:** 849–851, 1977.
31. Moingeon, P., J. M. Bidart, G. F. Alberici and C. Bohuon. Characterization of a peripheral-type benzodiazepine binding site on human circulating lymphocytes. *Eur J Pharmacol* **92:** 147–149, 1983.
32. Nagele, R. G., J. F. Pietrolungo, M. C. Kosciuk, H. Lee and F. J. Roisen. Diazepam inhibits the spreading of chick embryo fibroblasts. *Exp Cell Res* **143:** 153–162, 1983.
33. Niehoff, D. L., R. D. Mashal, W. D. Horst, R. A. O'Brien, J. M. Palacios and M. J. Kuhar. Binding of a radiolabeled triazolopyridazine to a subtype of benzodiazepine receptor in the rat cerebellum. *J Pharmacol Exp Ther* **221:** 670–675, 1982.
34. Niehoff, D. L. and P. J. Whitehouse. Multiple benzodiazepine receptors: autoradiographic localization in normal human amygdala. *Brain Res* **276:** 237–245, 1983.
35. Patel, J. and P. J. Marangos. Differential effects of GABA on peripheral and central type benzodiazepine binding sites in the brain. *Neurosci Lett* **30:** 157–160, 1982.
36. Pellow, S. and S. E. File. Behavioral actions of Ro5-4864: a peripheral-type benzodiazepine? *Life Sci* **35:** 229–246, 1984.
37. Richards, J. G., H. Mohler and W. Haefely. Benzodiazepine binding sites: receptors or acceptors? *Trends Pharmacol Sci* **3:** 223–229, 1982.
38. Sieghart, W. Association of proteins irreversibly labeled by ^{3}H-flunitrazepam with different benzodiazepine receptors. *J Neural Transm* **Suppl 18:** 345–352, 1983.
39. Sieghart, W. Several new benzodiazepines selectively interact with a benzodiazepine receptor subtype. *Neurosci Lett* **38:** 73–78, 1983.
40. Sieghart, W. and G. Drexler. Irreversible binding of ^{3}H-flunitrazepam to different proteins in various brain regions. *J Neurochem* **41:** 47–55, 1983.
41. Sieghart, W., A. Mayer and G. Drexler. Properties of ^{3}H-flunitrazepam binding to different benzodiazepine binding proteins. *Eur J Pharmacol* **88:** 291–299, 1983.
42. Sieghart, W. and A. Schuster. Affinity of various ligands for benzodiazepine receptors in rat cerebellum and hippocampus. *Biochem Pharmacol* **33:** 4033–4038, 1984.
43. Squires, R. F., D. I. Benson, C. Braestrup, J. Coupet, C. A. Klepner, V. Myers and B. Beer. Some properties of brain-specific benzodiazepine receptors: new evidence for multiple receptors. *Pharmacol Biochem Behav* **10:** 825–830, 1979.
44. Squires, R. F. and C. Braestrup. Benzodiazepine receptors in rat brain. *Nature* **266:** 732–734, 1977.
45. Tenen, S. S. and J. D. Hirsch. B-carboline-3-carboxylic acid ethyl ester antagonizes diazepam activity. *Nature* **288:** 609–619, 1980.
46. Wang, J. K. T., J. I. Morgan and S. Spector. Benzodiazepines that bind at peripheral sites inhibit cell proliferation. *Proc Natl Acad Sci USA* **81:** 753–756, 1984.
47. Williams, M. Anxioselective anxiolytics. *J Med Chem* **26:** 619–628, 1983.
48. Yamamura, H. I., T. Mimaki, S. H. Yamamura, W. D. Horst, M. Morelli, G. Bautz and R. A. O'Brien. ^{3}H-CL 218,872, a novel triazolopyridazine which labels the benzodiazepine receptor in rat brain. *Eur J Pharmacol* **77:** 351–354, 1982.
49. Young, W, S., III and M. J. Kuhar. Autoradiographic localization of benzodiazepine receptors in the brains of humans and animals. *Nature* **280:** 393–395, 1979.
50. Young, W. S., III, D. Niehoff, M. J. Kuhar, B. Beer and A. Lippa. Multiple benzodiazepine receptor localization by light microscopic radiohistochemistry. *J Pharmacol Exp Ther* **216:** 425–430, 1981.
51. Zbinden, G. and L. O. Randall. Pharmacology of benzodiazepines: Laboratory and clinical correlations. *Adv Pharmacol* **5:** 213–291, 1967.