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# Characterization of [$^3$H]Alprazolam Binding to Central Benzodiazepine Receptors

R. TYLER McCABE,[1] DONALD R. MAHAN,* RANDALL B. SMITH* AND JAMES K. WAMSLEY[2]

*Neuropsychiatric Research Institute, 120 Eighth Street South, Fargo, ND 58103*
*Western Institute of Neuropsychiatry, Salt Lake City, UT 84108*
*and *The Upjohn Company, Kalamazoo, MI 49001*

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McCABE, R. T., D. R. MAHAN, R. B. SMITH AND J. K. WAMSLEY. *Characterization of [$^3$H]alprazolam binding to central benzodiazepine receptors.* PHARMACOL BIOCHEM BEHAV **37**(2) 365–370, 1990.—The binding of the triazolobenzodiazepine [$^3$H]alprazolam was studied to characterize the in vitro interactions with benzodiazepine receptors in membrane preparations of rat brain. Studies using nonequilibrium and equilibrium binding conditions for [$^3$H]alprazolam resulted in high specific to nonspecific (signal to noise) binding ratios. The binding of [$^3$H]alprazolam was saturable and specific with a low nanomolar affinity for benzodiazepine receptors in the rat brain. The $K_d$ was 4.6 nM and the $B_{max}$ was 2.6 pmol/mg protein. GABA enhanced [$^3$H]alprazolam binding while several benzodiazepine receptor ligands were competitive inhibitors of this drug. Compounds that bind to other receptor sites had a very weak or negligible effect on [$^3$H]alprazolam binding. Alprazolam, an agent used as an anxiolytic and in the treatment of depression, acts in vitro as a selective and specific ligand for benzodiazepine receptors in the rat brain. The biochemical binding profile does not appear to account for the unique therapeutic properties which distinguish this compound from the other benzodiazepines in its class.

Benzodiazepine receptors Receptor binding Triazolobenzodiazepines Alprazolam Rat brain membranes

CHARACTERIZATION of receptors which specifically bind benzodiazepines has been well-documented (42, 46, 59, 61, 66). In the central nervous system, these agents are widely used therapeutically for their anxiolytic, anticonvulsant and sedative-hypnotic properties (33, 34, 63). The benzodiazepines produce these central pharmacological actions by potentiation of the postsynaptic membrane response to gamma-aminobutyric acid (GABA) on an oligomeric receptor complex (21, 27, 28, 45, 48, 62). When the benzodiazepine receptors are activated, GABA is released into the synaptic cleft and exposed to the postjunctional membrane where an alteration in chloride ion conductance occurs (24,52). The chloride ionophores open in response to GABA receptor stimulation (31), resulting in chloride influx down the concentration gradient, and ultimately in neuronal inhibition of the affected cell. Benzodiazepine actions are thought to be mediated via allosteric linkage of their receptors to low affinity $GABA_A$ receptors (47,65), which increase the frequency of chloride ion channel opening (5,67).

Many benzodiazepines appear to be indistinguishable on the basis of in vitro binding studies, although there are some differences. For instance, peripheral sites appear to exist which are different than the central type of receptors (7,8). Novel and therapeutically useful benzodiazepine compounds have been developed that interact with central and/or peripheral benzodiazepine receptor sites (63). Both flunitrazepam and diazepam recognize central and peripheral sites while Ro15-1788 and clonazepam are more selective for central receptors (4, 8, 18, 37, 41, 51, 63). The binding of tritiated agents to central benzodiazepine receptors has been studied extensively and the distribution of these sites has been established autoradiographically (45,69). These central receptors appear to be represented by two putative subtypes which have been designated as benzodiazepine-1 and benzodiazepine-2 receptors (BZ-1 and BZ-2). Many agents such as flurazepam, clonazepam, diazepam and Ro15-1788 do not discriminate between these heterogeneous receptor populations. However, quazepam, 2-oxo-quazepam (6, 15, 68, 70), the triazolopyridazine CL 218,872 (29,43) and the β-carbolines (44) are more selective for BZ-1 sites. These receptor subtypes may relate to two different conformations of the same receptor in the brain (38) or distinct receptor subtypes produced as different gene products (44,46). Also, binding to the peripheral benzodiazepine receptor sites has been analyzed with [$^3$H]Ro5-4864 (51) and these sites have been localized in the brain as well as the periphery (3,26).

Triazolobenzodiazepines appear to differ from other benzodi-

[1]Present address: Laboratory of Neuroscience, National Institute of Diabetes, Digestive and Kidney Diseases, Building 8, Room 111, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892.

[2]Requests for reprints should be addressed to James K. Wamsley, Ph.D., Director of Research, Neuropsychiatric Research Institute, 120 Eighth Street South, Fargo, ND 58103.

azepines with regard to their in vivo characteristics (57,60). The triazolobenzodiazepine alprazolam is a clinically effective drug prescribed for its unique properties (12,17). Alprazolam has been reported to possess anxiolytic (1, 14, 36) and antidepressant (22, 23, 39, 49, 50) activity. Additionally, this compound has been useful in the treatment of panic disorder or agoraphobia with panic attack (2, 9, 11, 20, 32, 55). The availability of [$^3$H]alprazolam has allowed the in vitro characterization of binding to central benzodiazepine receptors in membrane preparations of the rat brain. In the present study, we report findings from nonequilibrium, equilibrium saturation, and displacement studies of [$^3$H]alprazolam binding to benzodiazepine receptors. The binding to benzodiazepine receptor subtypes and other unrelated sites was analyzed to determine if the unique properties of alprazolam could be accounted for on the basis of receptor selectivity.

## METHOD

Male Sprague-Dawley rats were housed under a 12-hr light/dark cycle and allowed to consume fresh food and water ad lib. The animals were deeply anesthetized with chloroform or $CO_2$, and the brains were rapidly excised. Membranes from whole brain, cortex, nucleus accumbens, or hippocampus were prepared as described. Whole brains were homogenized in 20 volumes of 0.32 M sucrose (4°C) using a Teflon pestle in a glass tube. The homogenates were centrifuged at 3,000 rpm for 10 min. Subsequently, the crude pellets ($P_1$) were discarded and supernatants resuspended in sucrose and centrifuged for 45 min at 150,000 × g (Beckman rotor 50.2 Ti). The supernatants were decanted and the pellets ($P_2$) resuspended in distilled-deionized water to osmotically rupture the cells and release endogenous GABA and any other substances that may interfere with subsequent binding. A second centrifugation was performed at 150,000 × g for 30 min. Again, the supernatants were discarded and pellets resuspended in 50 mM Tris-HCl buffer (pH 7.4 at 4°C) and centrifuged a final time at 150,000 × g for 15 min. The mitochondrial, microsomal and synaptosomal fractions were used without further purification. In additional experiments, isolated cortical, nucleus accumbens, and hippocampal tissues were homogenized with a Brinkmann Polytron (setting 6–7, 15 sec) in 20 volumes of 50 mM Tris-HCl buffer (pH 7.4 at 4°C). Lysed tissues were centrifuged at 23,000 × g for 20 min at 4°C. The pellets were resuspended in an equal volume of buffer and recentrifuged. This "washing" procedure was performed four times. Pellets were resuspended in 50 volumes of buffer for binding assays. Triplicate samples were used in all experiments. Protein content was determined by the method described by Lowry *et al.* (35).

Association studies were accomplished by incubating whole brain membranes in 50 mM Tris-HCl buffer (4°C) containing 3.0 nM [$^3$H]alprazolam (specific activity 15–45 Ci/mmol; provided by the Upjohn Company) for 1–80 min. Nonspecific binding was defined by 10 μM clonazepam. All binding reactions were terminated by rapid filtration through Whatman GF/B filters using a Brandel (Gaithersburg, MD) cell harvester followed by 4 × 2-ml rinses in buffer (4°C). Bound [$^3$H]alprazolam was determined by liquid scintillation counting. Saturation studies were performed by incubating membranes in various concentrations (0.15–26 nM of [$^3$H]alprazolam.

Modulation studies to assess the specificity and selectivity of [$^3$H]alprazolam (1.0–2.0 nM) binding to whole brain or cortical membranes were performed using various concentrations (0.5 nM–100 μM) of ethyl β-carboline-3-carboxylate (βCCE), quazepam, bicuculline methiodide, clonazepam, Ro15-1788, Ro5-4864, PK 11195, picrotoxin, triazolam or alprazolam. Additionally, the interaction of unrelated ligands with [$^3$H]alprazolam (3.0 nM) binding to whole brain, cortical, and hippocampal membranes was studied using various concentrations (1.0 nM–100 μM) of imipramine, desipramine and amitriptyline (antidepressants), clonidine, propranolol, WB4101, piperoxane and idazoxane (adrenergic ligands), baclofen ($GABA_B$ agonist), naloxone (opiate antagonist), glycine and strychnine (glycine receptor ligands), atropine (muscarinic ligand), buspirone and fenfluramine (serotonin receptor ligands), histamine, MK-801 (phenyclidine and excitatory amino acid receptor ligand), reserpine and amphetamine (amine uptake site ligands), and dopamine and sulpiride (dopamine receptor ligands). The binding of [$^3$H]alprazolam (4.0 nM) to membranes of nucleus accumbens tissue was studied in the presence of each of the following drugs (1.0 μM) with and without the addition of spiperone (1.0 μM): Ro15-1788, sulpiride, desipramine, chlorimipramine, d-amphetamine, alprazolam, and serotonin. Incubations with nucleus accumbens membranes were performed using 50 mM Tris-HCl (pH 7.4, 4°C) buffer containing: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% ascorbic acid; or 120 mM NaCl, 100 nM pargyline, and 0.001% ascorbic acid.

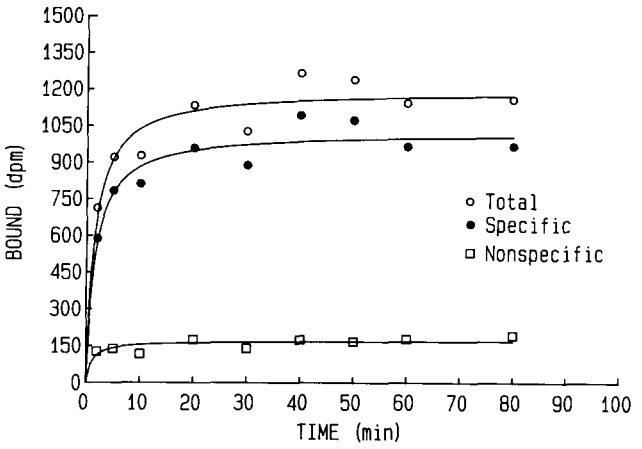


FIG. 1. Association of [$^3$H]alprazolam binding to membranes of whole rat brain. Tissues were prepared and assayed as described in the Method section. The binding of [$^3$H]alprazolam (3.0 nM) was performed by varying the period (1–80 min) of incubation in buffer (4°C) containing the tritiated ligand. Equilibrium was reached within 10 minutes of incubation. A 45-min incubation was arbitrarily selected for use in subsequent assays. Values are from two separate assays and represent means from triplicate samples.

## RESULTS

The binding of [$^3$H]alprazolam to benzodiazepine receptors associated fairly rapidly and reached equilibrium by 45 min. Figure 1 illustrates the association curve for [$^3$H]alprazolam binding to whole brain membranes and demonstrates that specific binding was approximately 85%. Thus, in all subsequent experiments, membranes were incubated in 50 mM Tris-HCl buffer (pH 7.4 at 4°C) for 45 min. Saturation experiments of [$^3$H]alprazolam binding yielded a $K_d$ of 4.6 nM and $B_{max}$ of 2.6 pmol/mg protein. Thus, [$^3$H]alprazolam binding to benzodiazepine receptors was saturable and of high affinity. Figure 2 shows the saturation isotherm for [$^3$H]alprazolam binding.

The order of potency for competitive inhibition of [$^3$H]alprazolam binding to benzodiazepine sites in whole brain membranes was: clonazepam > triazolam > alprazolam > Ro15-1788 > βCCE > quazepam. These data are shown in Table 1 and

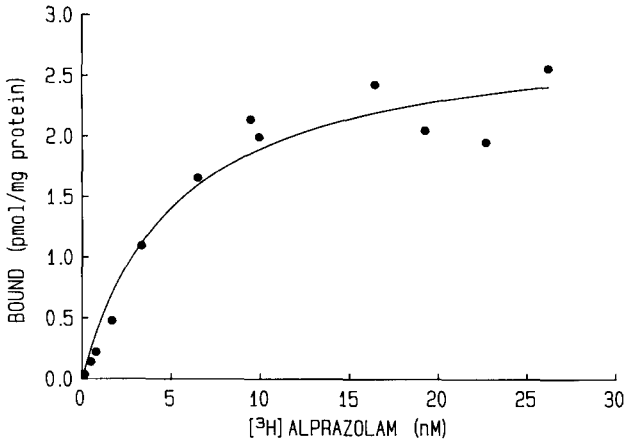


FIG. 2. Saturation isotherm of [$^3$H]alprazolam binding. Membranes (whole brain) were incubated for 45 min in buffer (4°C) containing various concentrations (0.15–26 nM) of [$^3$H]alprazolam. The reaction was followed by rapid filtration and 4 × 2-ml rinses in ice-cold fresh buffer. Data represent two separate experiments and are means of triplicate samples. The saturation isotherm yielded a $K_d$ of approximately 4.6 nM and $B_{max}$ of 2.6 pmol/mg protein.

inhibition curves are illustrated in Fig. 3. Displacement of [$^3$H]alprazolam binding to cortical membranes by benzodiazepine receptor ligands yielded the following order of potency: clonazepam > triazolam > Ro15-1788 > alprazolam > βCCE > quazepam (Table 1). Ro5-4864 and PK 11195 did not displace [$^3$H]alprazolam binding until very high concentrations were attained. GABA added to the incubation medium enhanced [$^3$H]alprazolam binding 230% (1 mM) in whole brain, 126% (100 mM) in cortex, and 130% (100 mM) in hippocampus. The results obtained with other ligands indicated that [$^3$H]alprazolam is a very weak competitor for these other sites (if at all) and demonstrated [$^3$H]alprazolam behaves as a typical benzodiazepine receptor ligand. The $K_i$ values for these unrelated agents are shown in Table 1. Modulation studies of [$^3$H]alprazolam binding to dissected regions of cortex, nucleus accumbens, and hippocampus did not show significant inhibition of radioligand binding. The binding of some of these ligands is sodium dependent, thus, incubations were performed in the presence or absence of 120 mM NaCl. Specific binding of [$^3$H]alprazolam (3.0 nM) was not significantly altered by including 120 mM NaCl in the incubation medium (data not shown).

TABLE 1

MODULATION [$^3$H]ALPRAZOLAM BINDING

| Drug | [$^3$H]Alprazolam Inhibition Cortex, $K_i$ (nM) | Whole Brain, $K_i$ (nM) |
|---|---|---|
| BZ Receptor Related: | | |
| alprazolam | 3.9 ± 0.2 | 5.5 ± 1.5 |
| βCCE | 6.0 ± 0.3 | 14.5 ± 3.7 |
| bicuculline methiodide | 14,000 | — |
| clonazepam | 1.0 ± 0.2 | 2.8 ± 0.3 |
| PK 11195 | 14,000 | >1,000,000 |
| picrotoxin | 22,000 | — |
| quazepam | 129 ± 18 | 325 ± 12 |
| Ro15-1788 | 2.1 ± 0.1 | 7.8 ± 2.1 |
| Ro5-4864 | >100,000 | 64,000 |
| triazolam | 1.1 ± 0.04 | 7.0 ± 0.6 |
| BZ Receptor Unrelated: | | |
| amitriptyline | >100,000 | — |
| amphetamine | >100,000 | — |
| atropine | >100,000 | — |
| baclofen | >100,000 | >1,000,000 |
| buspirone | >100,000 | — |
| clonidine | >100,000 | >1,000,000 |
| desipramine | >100,000 | >100,000 |
| dopamine | >100,000 | — |
| fenfluramine | >100,000 | — |
| glycine | >100,000 | — |
| histamine | >100,000 | — |
| idazoxan | — | >1,000,000 |
| imipramine | >100,000 | 14,000 |
| MK-801 | >100,000 | — |
| naloxone | >100,000 | — |
| piperoxane | >100,000 | — |
| propranolol | >100,000 | >1,000,000 |
| reserpine | >100,000 | — |
| strychnine | >100,000 | — |
| sulpiride | — | 7,600 |
| WB 4101 | >100,000 | — |

Modulation of [$^3$H]alprazolam binding in rat membrane preparations. Tissues were prepared and assayed as described in the Method section. Membranes were incubated in buffer containing [$^3$H]alprazolam and differing concentrations of the agents listed. $K_i$ (nM) values determined by the equation $K_i = IC_{50}/1 + [L]/K_D$. Values (mean ± SEM) are from three separate experiments and represent triplicate samples from each assay.

## DISCUSSION

The binding of [$^3$H]alprazolam is shown to be saturable, specific, selective, and of high affinity for benzodiazepine receptors. [$^3$H]Alprazolam associated rapidly and saturated a finite receptor population when reaching a concentration of 10 nM. The $K_d$ and $B_{max}$ values were 4.6 nM and 2.6 pmol/mg protein, respectively. The equilibrium dissociation constant ($K_d$) of alprazolam was consistent with reported $IC_{50}$ values for displacement of benzodiazepine ligand binding (13). The $B_{max}$ values obtained using [$^3$H]alprazolam agree well with previous estimates for benzodiazepine compounds (10, 25, 33, 64). GABA enhanced [$^3$H]alprazolam binding to membrane preparations (whole brain, cortex, and hippocampus) and this property was similar to other benzodiazepines.

The hypothesis that the binding of alprazolam does not discriminate between BZ-1 and BZ-2 receptors is consistent with the results observed in the present study in which nonsubtype-selective agents such as clonazepam, triazolam, alprazolam, and Ro15-1788 were more potent for displacing [$^3$H]alprazolam binding from benzodiazepine receptors than agents that preferentially recognize BZ-1 receptor sites (i.e., βCCE and quazepam). Additionally, [$^3$H]alprazolam appeared to be weakly inhibited by the $GABA_A$ receptor antagonist bicuculline methiodide and the convulsant compound picrotoxin (Table 1). A wide variety of other agents did not modulate or were very weak competitors of [$^3$H]alprazolam binding in vitro.

It has been reported that potentiated aggression induced by clonidine is completely abolished by alprazolam (30). Also, concomitant administration of an $alpha_2$-adrenergic receptor antagonist (i.e., idazoxan) markedly potentiates the anticonflict effects of alprazolam (60). One might speculate that alprazolam may be interacting with the $alpha_2$-adrenergic receptor in order to

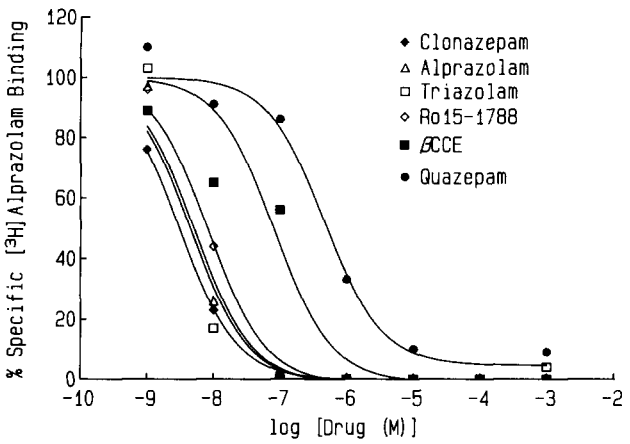


FIG. 3. Competition curves for displacement of [$^3$H]alprazolam binding. Displacement of [$^3$H]alprazolam binding (1.0 nM) to whole rat brain membranes was studied using 1 nM–1 mM of each drug. Data (mean % specific binding) are from triplicate samples and represent three separate experiments. $K_i$ (nM) values in descending order of potency are: clonazepam (2.8 ± 0.3), triazolam (5.5 ± 1.5), alprazolam (7.0 ± 0.6), Ro15-1788 (7.8 ± 2.1), βCCE (15 ± 3.7), and quazepam (325 ± 12). The graphs show mass action displacement of the various compounds which differ in their potencies, but do not indicate recognition of multiple sites.

have these effects. Results obtained in the present study (attempts to displace [$^3$H]alprazolam with clonidine, idazoxan, and piperoxane) would indicate that if some modulation is occurring, it is not a direct effect on the binding of the benzodiazepine and it is not apparent in vitro. Other reports suggest a potential association of alprazolam with beta-adrenergic receptors (53, 54, 56). For instance, administration (2–3 weeks duration) of alprazolam is capable of attenuating a reserpine-induced increase in beta-adrenergic receptors (54). This would not seem to be a direct receptor-mediated effect since the beta-receptor antagonist propranolol did not interfere appreciably with alprazolam binding. Other indirect studies have indicated that alprazolam may be interacting with dopamine type-2 receptors in isolated brain areas labeled with [$^3$H]sulpiride (16). This observation required the dissection of small brain regions (amygdala and nucleus accumbens) in order to see the effect. Again, our studies with [$^3$H]alprazolam did not show an interaction in the nucleus accumbens. Autoradiographic studies may be required to verify any selective effect in a very small region.

Receptor binding studies performed in vitro, which utilize benzodiazepine ligands, are usually incubated at 4°C (58). It has been shown that many ligands have a higher affinity and lower rate of dissociation at lower temperatures (19). These observations appear to apply to [$^3$H]alprazolam binding. Furthermore, the binding of this triazolobenzodiazepine is not different from other benzodiazepines. However, alprazolam may have different effects in vivo (40) that cannot be accounted for on the basis of its in vitro binding characteristics.

The present results indicate that [$^3$H]alprazolam possesses properties similar to other central benzodiazepine agonists that recognize both BZ receptor subtypes in the central nervous system. The binding is saturable, specific, selective, and of high affinity (recognizing benzodiazepine receptors with a $K_d$ in the low nanomolar range). Alprazolam is an excellent probe for defining benzodiazepine receptors in the central nervous system and its use may prove to be important in future studies aimed at investigating these receptors and their pharmacological effects. The binding profile of alprazolam does not seem to be distinguishable from that of other benzodiazepines and does not account for the unique therapeutic properties of alprazolam (use of the drug in the treatment of panic disorder or depression).

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## REFERENCES

1. Aden, G. C.; Thein, S. G., Jr. Alprazolam compared to diazepam and placebo. J. Clin. Psychiatry 41:245–248; 1980.
2. Alexander, P. E.; Alexander, D. D. Alprazolam treatment for panic disorders. J. Clin. Psychiatry 47:301–304; 1986.
3. Anholt, R. R. H.; DeSouza, E. B.; Oster-Granite, M. L.; Snyder, S. H. Peripheral-type benzodiazepine receptors: Autoradiographic localization in whole-body sections of neonatal rats. J. Pharmacol. Exp. Ther. 233:517–526; 1985.
4. Anholt, R. R. H.; Murphy, K. M. M.; Mack, G. E.; Snyder, S. H. Peripheral type benzodiazepine receptors in the central nervous system: Localization to olfactory nerves. J. Neurosci. 4:593–603; 1984.
5. Barker, J. G.; Gratz, E.; Owen, D. G.; Study, R. E. Pharmacological effects of clinically important drugs on the excitability of cultured mouse spinal neurons. In: Bowery, N. G., ed. Actions and interactions of GABA and benzodiazepines. New York: Raven Press; 1984:203–216.
6. Billard, W.; Crosby, G.; Iorio, L.; Chipkin, R.; Barnett, A. Selective affinity of the benzodiazepines quazepam and 2-oxo-quazepam for BZ1 binding site and demonstration of $^3$H-2-oxo-quazepam as a BZ1 selective radioligand. Life Sci. 42:179–187; 1987.
7. Braestrup, C.; Squires, R. F. Benzodiazepine receptors in rat brain. Nature 266:732–734; 1977.
8. Braestrup, C.; Squires, R. F. Specific benzodiazepine receptors in rat brain characterized by high affinity [$^3$H]diazepam binding. Proc. Natl. Acad. Sci. USA 74:3805–3809; 1977.
9. Charney, D. S.; Heninger, G. R. Noradrenergic function and the mechanism of action of antianxiety treatment. Arch. Gen. Psychiatry 42:458–467; 1985.
10. Chiu, T. H.; Rosenberg, H. C. Comparison of the kinetics of [$^3$H]diazepam and [$^3$H]flunitrazepam binding to cortical synaptosomal membranes. J. Neurochem. 39:1716–1725; 1982.
11. Chouinard, G.; Annable, L.; Fontaine, R.; Solyom, L. Alprazolam in the treatment of generalized anxiety and panic disorders: A double blind placebo-controlled study. Psychopharmacology (Berlin) 77: 229–233; 1982.
12. Ciraulo, C. A.; Barnhill, J. G.; Boxenbaum, H. G.; Greenblatt, D. J.; Smith, R. B. Pharmacokinetics and clinical effects of alprazolam following single and multiple oral doses in patients with panic disorder. J. Clin. Pharmacol. 26:292–298; 1986.
13. Clow, A.; Glover, V.; Sandler, M. Triazolam, an anomalous benzodiazepine receptor ligand: In vitro characterization of alprazolam and triazolam binding. J. Neurochem. 45:621–625; 1985.
14. Cohn, J. B. Multicenter double-bind efficacy and safety study comparing alprazolam, diazepam and placebo in clinically anxious patients. J. Clin. Psychiatry 42:347–351; 1981.
15. Corda, M. G.; Giorgi, O.; Longoni, B.; Ongini, E.; Montaldo, S.; Biggio, G. Preferential affinity of $^3$H-2-oxo-quazepam for type 1 benzodiazepine recognition sites in the human brain. Life Sci. 42:189–197; 1987.
16. Csernansky, J. G.; Csernansky, C. A.; Hollister, L. E. [$^3$H]-Sulpiride labels mesolimbic non-dopaminergic sites that bind antidepressant

drugs. Experientia 41:1419–1421; 1985.
17. Dawson, G. W.; Jue, S. G.; Brogden, R. N. Alprazolam: A review of its pharmacodynamic properties and efficacy in the treatment of anxiety and depression. Drugs 27:132–147; 1984.
18. De Souza, E. B.; Anholt, R. R. H.; Murphy, K. M. M.; Snyder, S. H.; Kuhar, M. J. Peripheral-type benzodiazepine receptors in endocrine organs: Autoradiographic localization in rat pituitary, adrenal and testis. Endocrinology 116:567–573; 1985.
19. Doble, A. Comparative thermodynamics of benzodiazepine receptor ligand interactions in rat neuronal membranes. J. Neurochem. 40: 1605–1612; 1983.
20. Dunner, D. L.; Ishiki, D.; Avery, D. H.; Wilson, L. G.; Hyde, T. H. Effect of alprazolam and diazepam on anxiety and panic attacks in panic disorder: A controlled study. J. Clin. Psychiatry 47:458–460; 1986.
21. Enna, S. J. The GABA receptors. Clifton, NJ: Humana Press; 1983.
22. Feighner, J. P. Open-label study of alprazolam in severely depressed inpatients. J. Clin. Psychiatry 44:332–334; 1983.
23. Feighner, J. P.; Aden, G. C.; Fabre, L. F.; Rickels, K.; Smith, W. T. Multiclinic double-blind safety and efficacy comparison of alprazolam, imipramine, and placebo in the treatment of depression. JAMA 249:3057–3064; 1983.
24. Gallager, D. W.; Tallman, J. F. Consequence of benzodiazepine receptor occupancy. Neuropharmacology 22:1493–1498; 1983.
25. Gee, K. W.; Yamamura, H. I. Photoaffinity labeling of benzodiazepine receptors in rat brain with flunitrazepam alters the affinity of benzodiazepine receptor agonist but not antagonist binding. J. Neurochem. 41:1407–1413; 1983.
26. Gehlert, D. R.; Yamamura, H. I.; Wamsley, J. K. Autoradiographic localization of "peripheral" benzodiazepine binding sites in the rat brain, heart and kidney. Naunyn Schmiedebergs Arch. Pharmacol. 328:454–460; 1985.
27. Haefely, W. The biological basis of benzodiazepine actions. The benzodiazepines today—two decades of research and clinical experience. J. Psychoactive Drugs 15:19–39; 1983.
28. Haefely, W.; Polc, P.; Schaffner, R.; Keller, H. H.; Pieri, L.; Mohler, H. Facilitation of GABAergic transmission by drugs. In: Krogsgaard-Larsen, P.; Scheel-Kruger, J.; Kofod, H., eds. GABA-neurotransmitters. Copenhagen: Munksgaard; 1979:357–375.
29. Klepner, C. A.; Lippa, A. S.; Benson, K. I.; Sano, M. C.; Beer, B. Resolution of two biochemically and pharmacologically distinct benzodiazepine receptors. Pharmacol. Biochem. Behav. 11:457–462; 1979.
30. Kostowski, W.; Valzelli, L.; Baiguena, G. Effect of chronic administration of alprazolam and adinazolam on clonidine- or apomorphine-induced aggression in laboratory rodents. Neuropharmacology 25: 757–761; 1986.
31. Krnjevic, K. Chemical nature of synaptic transmission in vertebrates. Physiol. Rev. 54:418–540; 1974.
32. Liebowitz, M. R.; Fryer, A. J.; Gorman, J. M.; Campeas, R.; Levin, A.; Davies, S. R.; Goetz, D.; Klein, D. F. Alprazolam in the treatment of panic disorders. J. Clin. Psychopharmacol. 6:13–20; 1986.
33. Lippa, A. S.; Critchett, D. J.; Sano, M. C.; Klepner, C. A.; Greenblatt, E. N.; Coupet, J.; Beer, B. Benzodiazepine receptors: Cellular and behavioral characteristics. Pharmacol. Biochem. Behav. 10:831–843; 1979.
34. Lippa, A. S.; Klepner, C. A.; Benson, D. I.; Critchett, D. J.; Sano, M. C.; Beer, B. The role of GABA in mediating the anticonvulsant properties of benzodiazepines. Brain Res. Bull. 5:861–865; 1979.
35. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275; 1951.
36. Maletzky, B. M. Anxiolytic efficacy of alprazolam compared to diazepam and placebo. J. Int. Med. Res. 8:139–143; 1980.
37. Marangos, P. J.; Patel, J.; Boulenger, J.-P.; Clark-Rosenberg, R. Characterization of peripheral-type benzodiazepine binding sites in brain using [$^3$H]Ro5-4864. Mol. Pharmacol. 22:26–32; 1982.
38. Martin, I. L.; Brown, C. L.; Doble, A. Multiple benzodiazepine receptors: Structures in the brain or structures in the mind? A critical review. Life Sci. 32:1925–1933; 1983.
39. Mendels, J.; Schless, A. P. Comparative efficacy of alprazolam, imipramine, and placebo administration once a day in treating depressed patients. J. Clin. Psychiatry 47:357–361; 1986.
40. Miller, L. G.; Greenblatt, D. J.; Barnhill, J. G.; Deutsch, S. I.; Shader, R. I.; Paul, S. M. Benzodiazepine receptor binding of triazolobenzodiazepines in vivo: Increased receptor number with low-dose alprazolam. J. Neurochem. 49:1595–1601; 1987.
41. Mohler, H.; Okada, T. C. Benzodiazepine receptors: Demonstration in the central nervous system. Science 198:849–851; 1977.
42. Müller, W. E. The benzodiazepine receptor: drug receptor only or a physiologically relevant part of our central nervous system? Cambridge: University Press; 1987.
43. Niehoff, D. L.; Marshal, R. D.; Horst, W. D.; O'Brien, R. A.; Palacios, J. M.; Kuhar, M. J. Binding of a radiolabeled triazolopyradazine to a subtype of benzodiazepine receptor in the rat cerebellum. J. Pharmacol. Exp. Ther. 221:670–675; 1982.
44. Nielsen, M.; Braestrup, C. Ethyl beta-carboline-3-carboxylate shows differential benzodiazepine receptor interaction. Nature 286:606–607; 1980.
45. Olsen, R. W. Drug interactions at the GABA receptor-chloride ionophore complex. Annu. Rev. Pharmacol. Toxicol. 22:245–277; 1982.
46. Olsen, R. W.; McCabe, R. T.; Wamsley, J. K. $GABA_A$ receptor subtypes: autoradiographic comparison of GABA, benzodiazepine, and convulsant binding sites in the rat central nervous system. J. Chem. Neuroanat. 3:59–76; 1990.
47. Olsen, R. W.; Snowhill, E. W.; Wamsley, J. K. Autoradiographic localization of low affinity GABA receptors with [$^3$H]bicuculline methochloride. Eur. J. Pharmacol. 99:247–248; 1984.
48. Olsen, R. W.; Venter, J. C. Benzodiazepine/GABA receptors and chloride channels: Structural and functional properties. New York: Alan R. Liss; 1986.
49. Pitts, W.; Fann, W.; Sajadi, C.; Snyder, S. Open-label study of alprazolam in older depressed inpatients. J. Clin. Psychiatry 44: 213–215; 1983.
50. Rickels, K.; Feighner, J. P.; Smith, W. T. Double-blind safety and efficacy study comparing alprazolam, amitriptyline, doxepin and placebo in the treatment of depression. Arch. Gen. Psychiatry 42:134–141; 1985.
51. Schoemaker, H.; Boles, R. G.; Horst, W. D.; Yamamura, H. I. Specific high affinity binding sites for [$^3$H]Ro5-4864 in rat brain and kidney. J. Pharmacol. Exp. Ther. 225:61–69; 1983.
52. Schofield, P. R.; Darlison, M. G.; Fujita, N.; Burt, D. R.; Stephenson, F. A.; Rodriguez, H.; Rhee, L. M.; Ramachandran, J.; Reale, V.; Glencorse, T. A.; Seeburg, P. H.; Barnard, E. A. Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor superfamily. Nature 320:221–227; 1987.
53. Sethy, V. H.; Hodges, D. H. Alprazolam in a biochemical model of depression. Biochem. Pharmacol. 31:3155–3157; 1982.
54. Sethy, V. H.; Hodges, D. H. Role of beta-adrenergic receptors in the antidepressant activity of alprazolam. Res. Commun. Chem. Pathol. Pharmacol. 36:329–332; 1982.
55. Sheehan, D. V.; Coleman, J. H.; Greenblatt, D. J.; Watkins, D.; Peterson, M.; Schildkraut, J. J.; Uzogava, E.; Orsulak, P. J.; Jones, K. J.; Levine, P. H. Some biological correlates of panic attacks with agoraphobia and their response to a new treatment. J. Clin. Psychopharmacol. 4:66–75; 1984.
56. Shehi, M.; Patterson, W. M. Treatment of panic attacks with alprazolam and propanolol. Am. J. Psychiatry 141:900–901; 1984.
57. Shepard, R. A. Neurotransmitters, anxiety and benzodiazepines: A behavioral review. Neurosci. Biobehav. Rev. 10:449–461; 1986.
58. Skolnick, P.; Schweri, M. M.; Williams, E. F.; Moncada, V. Y.; Paul, S. M. An in vitro binding assay which differentiates benzodiazepine "agonists" and "antagonists". Eur. J. Pharmacol. 78: 133–136; 1982.
59. Snyder, S. H. Drugs and neurotransmitter receptors. New perspectives with clinical relevance. JAMA 261:3126–3129; 1989.
60. Söderpalm, B.; Engel, J. A. $\alpha_2$-Adrenoceptor antagonists potentiate the anticonflict and the rotarod impairing effects of benzodiazepines. J. Neural Transm. 76:191–204; 1989.
61. Squires, R. F. Gaba and benzodiazepine receptors, vol. I. Boca Raton: CRC Press; 1988.
62. Study, R. E.; Barker, J. L. Diazepam and (−)-pentobarbital: Fluctuation analysis reveals different mechanisms for potentiation of gamma-aminobutyric acid response in cultured neurons. Proc. Natl.

Acad. Sci. USA 78:7180–7194; 1981.
63. Tallman, J. F.; Paul, S. M.; Skolnick, P.; Gallagher, D. W. Receptors for the age of anxiety: Pharmacology of the benzodiazepines. Science 207:274–281; 1980.
64. Trullas, R.; Havoundjian, H.; Skolnick, P. Stress-induced changes in t-[$^{35}$S]butylbicyclophosphorothionate binding to gamma-aminobutyric acid-gated chloride channels are mimicked by in vitro occupation of benzodiazepine receptors. J. Neurochem. 49:968–974; 1987.
65. Unnerstall, J. R.; Kuhar, M. J.; Niehoff, D. L.; Palacios, J. M. Benzodiazepine receptors are coupled to a subpopulation of GABA receptors: Evidence from a quantitative autoradiographic study. J. Pharmacol. Exp. Ther. 218:797–804; 1981.
66. Usdin, E.; Skolnick, P.; Tallman, J. F., Jr.; Greenblatt, D.; Paul, S. M. Pharmacology of benzodiazepines. London: Macmillan; 1982.
67. Vicini, S.; Mienville, J. M.; Costa, E. Actions of benzodiazepine and beta-carbolines on gamma-aminobutyric acid-activated Cl-channels recorded from membrane patches of neonatal rat cortical neurons in culture. J. Pharmacol. Exp. Ther. 243:1195–1201; 1987.
68. Wamsley, J. K.; Golden, J. S.; Yamamura, H. I.; Barnett, A. Autoradiographic demonstration of the selectivity of two 1-N-trifluoroethyl benzodiazepines for the BZD-1 receptors in the rat brain. Pharmacol. Biochem. Behav. 23:973–978; 1985.
69. Wamsley, J. K.; Palacios, J. M. Amino acid and benzodiazepine receptors. In: Bjorklund, A.; Hokfelt, T.; Kuhar, M. J., eds. Handbook of chemical neuroanatomy, vol. 3, Classical transmitters and transmitter receptors in the CNS. Amsterdam: Elsevier/North Holland; 1984:352–385.
70. Yezuita, J. P.; McCabe, R. T.; Barnett, A.; Iorio, L. C.; Wamsley, J. K. Use of the selective benzodiazepine-1 (BZ-1) ligand [$^{3}$H]2-oxo-quazepam (SCH 15-725) to localize BZ-1 receptors in the rat brain. Neurosci. Lett. 88:86–92; 1988.