

Benzodiazepine Receptor Binding of Benzodiazepine Hypnotics: Receptor and Ligand Specificity

LAWRENCE G. MILLER,¹ WENDY R. GALPERN,
JOHN J. BYRNES AND DAVID J. GREENBLATT

*Division of Clinical Pharmacology, Departments of Pharmacology and Psychiatry,
Tufts University School of Medicine and New England Medical Center, Boston, MA 02111*

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MILLER, L. G., W. R. GALPERN, J. J. BYRNES AND D. J. GREENBLATT. *Benzodiazepine receptor binding of benzodiazepine hypnotics: Receptor and ligand specificity*. PHARMACOL BIOCHEM BEHAV 43(2) 413-416, 1992. — Benzodiazepine (BDZ) hypnotics bind at a specific receptor located on postsynaptic neurons. Some data support specificity of binding for several hypnotics to receptor subtypes. We evaluated BDZ receptor binding in cerebral cortical membranes using agonist, antagonist, and subtype-specific ligands for commonly used hypnotics and their metabolites. All hypnotics competed similarly at BDZ₁ and BDZ₂ receptor subtypes except quazepam and its metabolite 2-oxo-quazepam and to a lesser extent hydroxyethyl flurazepam (EtOH) flurazepam. These compounds had relative specificity for the BDZ₁ site. Triazolam, estazolam, and flurazepam bound equally to sites labeled by agonists and antagonists but desalkylflurazepam, EtOH flurazepam, temazepam, quazepam, and 2-oxo-quazepam did not; in addition, these four compounds did not bind to the "peripheral" BDZ site labeled by Ro 5-4864. BDZ hypnotics differ in their receptor subtype and ligand binding characteristics.

Benzodiazepine Receptor Hypnotic

BENZODIAZEPINES (BDZs) constitute the majority of prescription hypnotic agents used in the United States. Currently available BDZs have been reported to differ in pharmacokinetics, including clearance, time of onset of action, and presence or absence of active metabolites (4,5). With regard to neurochemical characteristics, BDZs bind at a specific site located on the GABA_A receptor complex. Binding is usually performed using a radiolabeled BDZ agonist, and BDZs bind with differing apparent affinities (6). A BDZ antagonist, flumazenil, is also available, but few studies have addressed characteristics of binding with this ligand (6). In addition, a triazolopyridazine compound, CL 218872, has been reported to distinguish two BDZ receptor subtypes (designated BDZ₁ and BDZ₂) based upon differential affinities at these sites (14).

Some data support neurochemical differences among BDZ hypnotics. In particular, quazepam and one of its active metabolites, 2-oxo-quazepam, have been reported to bind with relative selectivity to the so-called BDZ₁ BDZ receptor subtype (1,12,15). To assess the BDZ receptor subtype selectivity of quazepam and other BDZ hypnotics, we determined effects

of these compounds on BDZ binding using the ligands flunitrazepam and flumazenil and the subtype-selective compound CL 218872.

METHOD

Materials

Male CD1 mice, 6-8 weeks of age (Charles River, Wilmington, MA) were maintained on a 12 L : 12 D cycle and given food and water ad lib. [³H]Flunitrazepam (FNTZ; spec. act. 70 Ci/mmol) and [³H]Ro 15-1788 (spec. act. 81 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Quazepam and 2-oxo-quazepam were obtained from Schering (Kenilworth, NJ). Flurazepam, desalkylflurazepam, and hydroxyethyl flurazepam (EtOH) flurazepam were gifts from Hoffmann-La Roche (Nutley, NJ). Triazolam was obtained from Upjohn (Kalamazoo, MI) and temazepam was obtained from Sandoz (East Hanover, NJ). CL 218872 was obtained from Lederle (Pearl River, NY). All other reagents were obtained from standard commercial sources.

¹ Requests for reprints should be addressed to Dr. Lawrence G. Miller, Box 1007, New England Medical Center, 750 Washington Street, Boston, MA 02111.

TABLE 1
K_i VALUES FOR HYPNOTIC DISPLACEMENT OF
AGONIST AND ANTAGONIST BDZ RECEPTOR BINDING

Compound	FNTZ K _i [nM]	Flumazenil K _i [nM]
Triazolam	0.9 ± 0.2	1.1 ± 0.2
Desalkylflurazepam	4.3 ± 0.6	11.9 ± 0.7
Estazolam	17.0 ± 0.7	24.6 ± 3.2
EtOH flurazepam	26.8 ± 0.8	111 ± 22.5
Flurazepam	34.8 ± 3.8	34.2 ± 3.0
Temazepam	41.0 ± 5.8	62.8 ± 7.3
Quazepam	250 ± 20	124 ± 22.5
2-oxo-Quazepam	276 ± 21	171 ± 18.8

K_i values were determined by displacement of 1 nM [³H]-FNTZ or [³H]flumazenil using 10⁻¹⁰-10⁻⁵ M of the displacing compounds. Results are mean ± SEM, n = 3 for each compound.

Methods

Drugs were dissolved in ethanol and diluted with binding buffer. In all cases, final ethanol concentrations in assays were 1% or less. The highest percentage of ethanol for each compound was used as vehicle. Binding was performed using mouse cortical synaptosomal preparations (P2) prepared as previously described (10). BDZ binding was performed as previously described (3). Equilibrium is achieved for both radioligands by 60 min (8), so this interval was used. Briefly, to duplicate or triplicate samples was added [³H]FNTZ, or [³H]flumazenil, 1nM. Samples were treated with buffer or clo-

TABLE 2
K_i VALUES FOR THE BDZ₁ SUBTYPE OF
BDZ RECEPTOR BINDING

Compound	FNTZ K _i With CL 218872 [nM]	Ratio of FNTZ K _i With and Without CL 218872
Triazolam	1.1 ± 0.2	1.22
Desalkylflurazepam	4.0 ± 1.3	0.93
Estazolam	21.8 ± 3.1	1.28
EtOH flurazepam	35.9 ± 2.2	1.34
Flurazepam	41.8 ± 4.7	1.20
Temazepam	41.5 ± 5.4	1.01
Quazepam	393 ± 27	1.57
2-oxo-Quazepam	412 ± 45	1.49

K_i values were determined by displacement of 1 nM [³H]-FNTZ in the presence of 1 μM CL 218872 using 10⁻¹⁰-10⁻⁵ M of the compounds. Results are mean ± SEM, n = 3 for each compound. For the ratios, mean K_i determined in the presence of CL 218872 is divided by mean K_i in the absence of CL 218872. Desalkylflurazepam is chemically identical to N-desalkyl-2-oxo-quazepam.

nazepam, 10⁻⁵ M, to assess total and nonspecific binding, respectively. BDZ hypnotics, 10⁻¹⁰ - 10⁻⁵ M, were added to additional samples. In some experiments, CL 218872 (10⁻⁶ M) was added to all samples. After incubation at 4°C for 60 min, samples were filtered using a Brandel M48R (Gaithersburg, MD) onto Whatman GF/B filters. Filters were washed twice with cold buffer and counted by scintillation spectrometry.

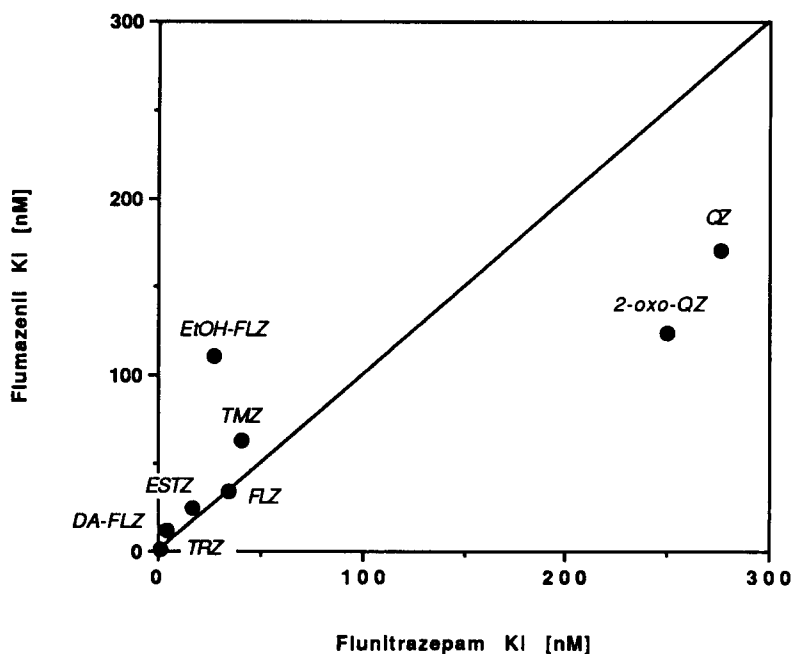


FIG. 1. Correlation of agonist and antagonist K_i values for hypnotics. Line is the line of identity. FNTZ, flunitrazepam; DA-FLZ, desalkylflurazepam; ESTZ, estazolam; EtOH FLZ, EtOH flurazepam; FLZ, flurazepam; QZ, quazepam; 2-oxo-QZ, 2-oxo-quazepam; TMZ, temazepam; TRZ, triazolam. Results are means of three determinations at each point. The correlation coefficient for these data is significant (r = 0.85, p < 0.05).

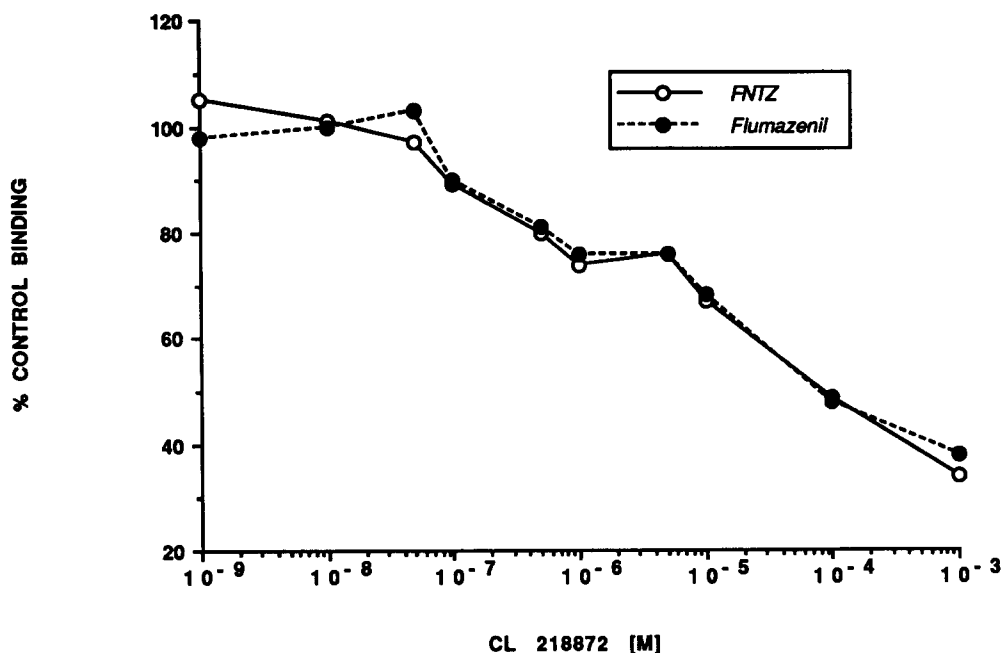


FIG. 2. Competition of CL 218872 using [³H]FNTZ and [³H]flumazenil. Binding was performed in cortical membranes at 4°C using 1 nM [³H]FNTZ or [³H]flumazenil. Results are from a representative experiment. FNTZ, flunitrazepam.

Assays were performed three times using a single cortical membrane. In a control experiment, a range of concentrations of CL 218872 was used with either radioligand to confirm that the 1/μM concentration of CL 218872 was adequate to antagonize BDZ₁ subtype binding.

Data Analysis

Data were analyzed using the EBDA programs (9). K_i values were calculated as described by Cheng and Prusoff (2).

RESULTS

Competition for BDZ receptor binding for BDZ hypnotics is presented in Table 1. Correlation of flunitrazepam and flumazenil competition was very high for triazolam, estazolam, and flurazepam. However, temazepam, desalkylflurazepam, and EtOH flurazepam decreased K_i for flunitrazepam compared to flumazenil, and quazepam and 2-oxo-quazepam increased K_i for flunitrazepam compared to flumazenil (Fig. 1).

Binding affinity of the various compounds for the BDZ₂ site is expressed as K_i in the presence of CL 218872 (Table 2). Relative affinity for the BDZ₁ site is expressed as the ratio of FNTZ K_i in the presence of CL 218872 divided by K_i in the absence of CL 218872 (Table 2). For all compounds except quazepam and 2-oxo-quazepam, K_i values for flunitrazepam in the presence and absence of CL 218872 are similar. This indicates similar affinity for both the BDZ₁ and BDZ₂ sites. For quazepam and 2-oxo-quazepam, K_i are significantly greater ($p < 0.05$) in the presence of CL 218872. This suggests a lower binding affinity for the BDZ₂ compared to the BDZ₁ site, indicating relative selectivity for the BDZ₁ site.

To determine whether the differences between agonist (FNTZ) and antagonist (flumazenil) binding for temazepam, EtOH flurazepam, and 2-oxo-quazepam might be due to dif-

ferences in binding at the "peripheral" BDZ site, competition experiments were performed with the specific peripheral ligand Ro 5-4864 for these compounds. K_i values for these compounds and desalkylflurazepam were all greater than 10⁻⁵ M, indicating no appreciable binding at the peripheral site (data not shown).

To confirm that 1 μM CL 218872 was adequate to antagonize binding at BDZ₁ sites, competition was performed using a range of concentrations of CL 218872 and either [³H]FNTZ or [³H]flumazenil in cortical membranes (Fig. 2). These data indicate a biphasic competition curve, with a plateau at approximately 75% of control indicating blockade of BDZ₁ sites. Concentrations of 1 μM CL 218872 appeared to block these sites for both radioligands, with concentrations of CL 218872 above 10 μM leading to binding at BDZ₂ sites also.

DISCUSSION

This study indicates differences in BDZ binding among hypnotics. As previously reported, K_i values for a series of hypnotic BDZs indicate high affinity for compounds such as triazolam and desalkylflurazepam and relatively low affinity for quazepam and 2-oxo-quazepam (1). Also consistent with previous reports, all compounds evaluated with the exception of quazepam and 2-oxo-quazepam had similar K_i in the presence and absence of the BDZ₁ subtype-specific ligand CL 218872 (1). This indicates similar affinity for the BDZ₁ and BDZ₂ binding sites. However, for quazepam and 2-oxo-quazepam the ratio of K_i with CL 218872 to K_i without CL 218872 was approximately 1.5, indicating reduced affinity in the presence of CL 218872 and conversely relative specificity for the BDZ₁ receptor subtype (13).

Binding was also evaluated using both agonist and antagonist radioligands. For triazolam, estazolam, and flurazepam, K_i values were similar with the two ligands. However, K_i for

desalkylflurazepam, EtOH flurazepam, and temazepam was greater using [³H]flumazenil than [³H]FNTZ, and for quazepam and 2-oxo-quazepam *K_i* was greater using [³H]FNTZ compared to [³H]flumazenil. FNTZ binds to both central and "peripheral" BDZ sites, the latter occurring on nonneuronal cells in brain; flumazenil binds only to central sites (6). It was possible that the enhanced binding with FNTZ compared to flumazenil for several compounds might have been due to binding at both peripheral and central sites. However, because these compounds did not appear to bind at the peripheral site this explanation is unlikely to be correct. More likely, possible differences in conformation of the agonist and antagonist BDZ binding sites account for the differences in binding observed using these ligands (6). The functional significance of such differential agonist and antagonist binding remains uncertain.

With regard to specific binding at BDZ receptor subtypes, this was determined indirectly by comparing FNTZ displacement in the presence and absence of excess CL 218872 to account for total binding and binding with BDZ₁ sites blocked, respectively (7). Of the compounds evaluated, only quazepam and 2-oxo-quazepam had substantially increased *K_i* values in the presence of CL 218872. Similar data have been reported in tissue and autoradiographic studies (1,11,15). These data indicate that the two compounds had a greater

overall affinity for the combination of BDZ₁ and BDZ₂ sites compared to affinity when BDZ₁ sites were blocked, indirectly indicating relative specificity for the BDZ₁ subtype. EtOH flurazepam also had a somewhat greater *K_i* value in the presence of CL 218872, suggesting that this compound may also have some selectivity for the BDZ₁ site.

In summary, our results indicate that most commonly used hypnotics bind to both BDZ₁ and BDZ₂ receptor subtypes with similar affinity, but that quazepam and 2-oxo-quazepam, and to a lesser extent EtOH flurazepam, exhibit some selectivity for the BDZ₁ subtype. In addition, desalkylflurazepam, EtOH flurazepam, temazepam, and 2-oxo-quazepam also appeared to have differential affinity for an agonist-labeled site compared to an antagonist-labeled site. These data indicate that hypnotics may differ in neurochemical properties. The functional significance of these distinctions remains to be determined.

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