[N-methyl-^{3}H]methylphenazepam, a New Ligand for Diazepin Receptors

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UDC 615.217:615.214.22]−092.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, No. 4, pp. 382−383, April, 1993
Original article submitted December 17, 1992

Key Words: *[N-methyl-^{3}H]methylphenazepam; [^{3}H]diazepam; phenazepam; benzodiazepin receptors;* GABA

In the study of the central benzodiazepin receptors, tritium-labeled 1,4-benzodiazepin, β-carbolines, and flumazenil are widely used [2,4,6]. The use of these compounds makes it possible to evaluate the affinity and density of the receptors, as well as to the study the effect on the process of binding of psychopharmacological preparations [2,7]. In this work we studied the patterns of specific binding of a new Russian-manufactured ligand of benzodiazepin receptors, [N-methyl-^{3}H]methylphenazepam, to synaptic membranes of the rat striatum.

MATERIALS AND METHODS

Experiments were carried out on white male rats weighing 170-200 g. Synaptic membranes of the striatum were isolated as described earlier [1]. The following three preparations were used in the radioligand analysis: [^{3}H]diazepam (Amersham, England); 2.85 TBc per mM), [^{3}H]diazepam (V. G. Khlopin Radium Institute, St. Petersburg; 2.3 TBc per mM), and [N-methyl-^{3}H]methylphenazepam (V. G. Khlopin Radium Institute, St. Petersburg; 2.4 TBc per mM). The incubation mixture contained 110-120 μg of membranes, 50 mM K-phosphate buffer, pH 7.4, and radioactive ligand in a concentration of 0.5-7 nM in a volume of 1 ml. Nonspecific binding was assessed in the presence of 30 mM diazepam (Sigma, USA). The samples were incubated for 1 hour at 0-3°C. Unbound label was discarded using GF/B filters (Whatman, England).

In the evaluation of the effect of GABA (Reanal, Hungary) and muscimol (Serva, Germany) on the binding of [^{3}H]diazepam and [N-methyl-^{3}H]methylphenazepam the ligand was used in a concentration of 3 nM. Here the incubation mixture contained in addition 150 mM NaCl. In the experiments on [N-methyl-^{3}H]methylphenazepam displacement, phenazepam was predissolved in DMSO and introduced in a volume of 10 μl. The radioactivity of the samples was measured on a Rackbeta 1217-802 counter. The parameters of ligand binding were determined according to the results of 3-5 similar individual experiments that were performed in double parallels. Calculations of K_d, B_{max}, and IC_{50} were performed using linear paired regression analysis according to the method of least squares on an ES-

TABLE 1. Parameters of Specific Binding of Benzodiazepin Receptor Ligands to Synaptic Membranes of Rat Striatum

Ligand	K_d, nM	B_{max}, fM per mg protein
[^{3}H]diazepam (Amersham)	5.5±0.7	163.2±10.0
[^{3}H]diazepam (Russia)	5.7±0.6	157.4±12.0
[N−methyl−^{3}H]methylphenazepam (Russia)	1.2±0.1*	177.2±21.0

Note: asterisk: $p<0.01$ when compared to K_d of imported specimen of [^{3}H]diazepam.

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0007-4888/93/0004-0404$12.50

TABLE 2. Effect of GABA–Tropic Preparations on Binding of Russian Ligands of Benzodiazepin Receptors to Synaptic Membranes of Rat Striatum

Preparation	[^{3}H]diazepam	[N-methyl-^{3}H]methylphenazepam
GABA	Enhancement, $EC_{50}=6.4\times10^{-7}$ M	No effect
Muscimol	Enhancement, $EC_{50}=5.1\times10^{-7}$ M	No effect
Diazepam		Inhibition, $IC_{50}=6.3\times10^{-7}$ M
Phenazepam		Inhibition, $IC_{50}=7.4\times10^{-8}$ M

Note: the GABA and muscimol concentration was in the range of $1\times10^{-8}-5\times10^{-4}$ M; the concentration of diazepam and phenazepam was $2.6\times10^{-12}-1.0\times10^{-5}$ M.

1841 computer. Protein content was determined after Lowry [5].

RESULTS

Table 1 presents the results on the specific binding of radioactive ligands to the membranes of the rat striatum. The values of receptor density as estimated using three ligands did not differ significantly. At the same time, the affinity of the [N-methyl-^{3}H]methylphenazepam receptors proved to be reliably higher than that of the others, this being reflected in a lower K_d value. Thus, [N-methyl-^{3}H]methylphenazepam can be classified among the high-affinity ligands of the benzodiazepin receptors. This preparation was also superior to both domestic and imported specimens of [^{3}H]diazepam in terms of the value of nonspecific binding. For instance, this parameter of labeled methylphenazepam was equal to 8-20% of the total binding, depending on the concentration. The corresponding values for both [^{3}H]diazepam preparations amounted to 35-40% of the total binding. A high level of radioligand nonspecific binding to membranes and/or filter may complicate the study [8,9].

Benzodiazepin binding to receptors is known to be enhanced by GABA [3]. Table 2 demonstrates this effect of GABA and muscimol on the reaction of Russian-made ligands. It was shown that agonists of $GABA_A$ receptors exert no influence on [N-methyl-^{3}H]methylphenazepam binding. However, [^{3}H]diazepam binding was enhanced in the presence of GABA and muscimol. This provides evidence that the [N-methyl-^{3}H]methylphenazepam binding sites are not modulated by $GABA_A$ agonist binding sites.

It was established that the inhibitory activity of phenazepam regarding [N-methyl-^{3}H]methylphenazepam binding exceeds that of diazepam 8.5 times (Table 2). Analysis of the data in the Linuiver-Barck coordinates revealed that both benzodiazepins competitively displaced the radioligand. However, one may assume that phenazepam has a greater affinity to [N-methyl-^{3}H]methylphenazepam binding sites.

Thus, [N-methyl-^{3}H]methylphenazepam was characterized by an increased affinity to benzodiazepin receptors as compared to the "traditional" ligand [^{3}H]diazepam. [N-methyl-^{3}H]methylphenazepam binding sites are not modulated by $GABA_A$ agonists and display a greater affinity to phenazepam than to diazepam.

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