Binding Characteristics of [3H]Quinupramine to Rat Brain Membrane Fractions

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Abstract—The binding characteristics of [3H]quinupramine to rat brain membrane fractions were studied. The specific binding of [3H]quinupramine to rat brain membrane fractions was stable, reversible and saturable. Scatchard analysis of the data from saturation experiments indicated that the specific binding was a single population with an affinity (K_D) of 3.04 nM, a maximal binding site number (B_max) of 714 fmol/mg protein, and a Hill coefficient (n_H) of 1.08. Compounds known to inhibit muscarinic cholinergic receptors such as atropine and quinuclidinyl benzilate were the most potent competitors of [3H]quinupramine binding. When the drug potencies in inhibiting [3H]quinupramine binding were tested in the presence of 10 nM atropine, mianserin was the most potent competitor. Studies of the subcellular fractions showed that there was an enrichment of [3H]quinupramine binding sites in the synaptosome fraction. The regional distribution study revealed the highest densities of binding sites in the cerebral cortex and the lowest in the cerebellum. Thus, the specific binding of [3H]quinupramine observed here can be accounted for by both muscarinic cholinergic and serotonin S_2 receptors.

The receptor binding assay has been considered as a useful technique for identifying the site of action for a variety of endobiotic and xenobiotic substances. For example, the discovery of high-affinity binding sites for psychoactive drugs (1-3) had opened up new approaches for the study of these drugs and their mechanisms of action. Furthermore, these procedures have been used to identify endogenous ligands for some drug binding sites. Recently, some investigators reported the characteristics of the specific binding sites of antidepressant drugs (4-13). Especially, the discovery of high affinity [3H]imipramine binding sites is of obvious interest, which suggests that an endogeneous "imipramine-like" substance may exist (14-16).

Quinupramine, (5-1-azabicyclo[2,2,2]oct-3-yl)-10,11-dihydro-5H-dibenzo[b,f]azepine, is a new potent antidepressant drug with a tricyclic dibenzazepine group as a moiety in the molecule. Quinupramine was a weak inhibitor of monoamine uptake systems (17), and it was found to possess decreased central serotonin S_2 receptor binding without altered β-adrenergic receptor binding after its repeated treatment (18). Through these observations, we have suggested that the pharmacological properties of quinupramine, chemically one of the typical tricyclic antidepressant drugs, are close to those of the atypical antidepressant drugs (18). In this paper, the studies were carried out to clarify the mechanisms of action of quinupramine by characterizing the specific binding sites in rat brain.

Materials and Methods

Animals: Male Sprague-Dawley rats (200–250 g) from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) were used...
throughout the experiments. The rats were housed under standard laboratory conditions with water and food ad libitum and light-dark cycles of 12 hr.

Preparation of tissue samples: After decapitation, the brains were rapidly removed and chilled in ice-cold saline. According to the method of Glowinski and Iversen (19), the specific regions were dissected over ice and were homogenized in appropriate volumes of ice-cold assay buffer (50 mM Tris-HCl, pH 7.7) with an Ultraturrax® (Junke & Kunkel KG, Staufen, West Germany) at 0°C for 15 sec. The homogenate was centrifuged at 50,000 x g for 10 min at 4°C, resuspended in the same buffer and centrifuged again at 50,000 x g for 10 min at 4°C. The final pellet was suspended in the buffer and stored on ice until use.

Subcellular fractionation: Various subcellular fractions of rat brain were obtained by differential and discontinuous sucrose gradient centrifugation (20). Whole brain without the cerebellum was homogenized in 10 volumes of ice-cold 0.32 M sucrose, using an Ultraturrax®. After centrifugation at 1,000 x g for 10 min at 4°C, the crude nuclear pellet (P1) was washed with an equal volume of 0.32 M sucrose. The crude mitochondrial pellet (P2) was obtained by centrifuging the combined supernatant fraction at 17,500 x g for 20 min at 4°C. The supernatant was centrifuged at 100,000 x g for 1 hr at 4°C to obtain the crude microsomal pellet (P3). Part of the P2 pellet was suspended in 0.32 M sucrose and further fractionated on a 0.8-1.2 M sucrose two-step discontinuous gradient.

[3H]Quinupramine binding assay: Routinely, incubation tubes received 0.1 ml of [3H]quinupramine (final concentration, 1.2 nM), 0.1 ml of various drugs, 0.3 ml of 50 mM Tris-HCl buffer (pH 7.7) and 0.5 ml of tissue suspension. For Scatchard analysis (21), tissue suspension was mixed with various concentrations of [3H]quinupramine (final concentration, 0.1 to 20 nM). Each assay was performed in duplicate. The final tissue concentration was 5 to 10 mg of wet weight tissue per ml. After incubation at 37°C for 15 min, the incubation mixtures were rapidly filtered under vacuum through glass fiber filters (GC-50, 0.5 μm, Toyo Roshi Co. Ltd., Tokyo, Japan), with 3×5 ml rinses of ice-cold assay buffer. The filters were dried and counted by a liquid scintillation spectrometer (Mark III, Searle Analytic, Des Plaines, IL, U.S.A.) in Biofluor® (NEN Research Products, Boston, MA, U.S.A.). The specific binding was determined as the difference between the total and the binding left in the presence of displacer (10 μM quinupramine or mequitazine).

Other receptor binding assay: Estimation of binding of the each ligand was carried out by the method of Sakamoto et al. (18, 22).

Protein amounts: Protein amounts of brain membrane fractions were determined by the modified Lowry method (23), using bovine serum albumin as the standard protein.

[3H]Ligands: [Imino-3H]quinupramine (10.2 Ci/mmol) was prepared by Amersham International (Buckinghamshire, England) by catalytic reduction of LM-2904, 5-(3-quinuclidinyl)-5H-dibenzo[b,f]azepine (Groupe Pharmuka, Gennevilliers, France), with tritium gas. The initiated compound was purified by thin-layer chromatography on silica gel. [3H]WB4101 (27 Ci/mmol), [phenyl-4-3H]clonidine hydrochloride (24 Ci/mmol), [di(4,6-phenyl-3H]dihydroalprenolol (71 Ci/mmol), [4,6-phenyl-3H]benzilate (42 Ci/mmol), [pyrindinyl-5-3H]pyrilamine hydrochloride (28 Ci/mmol) and [4,6-phenyl-3H]spiperone (17 Ci/mmol) were purchased from Amersham International. [N-Methyl-3H]imipramine hydrochloride (76.7 Ci/mmol) and [ethylene-3H]ketanserin hydrochloride (95 Ci/mmol) were from NEN Research Products (Boston, MA, U.S.A.).

Drugs: Chemicals were obtained from the following sources: quinupramine (Groupe Pharmuka and Nippon Shoji Kaisha, Ltd., Osaka, Japan), mequitazine and diazepam (Nippon Shoji Kaisha, Ltd.), imipramine hydrochloride, cimetidine and pyrilamine maleate (Sigma Chemical Co., St. Louis, MO, U.S.A.), amitriptyline hydrochloride (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan), desipramine hydrochloride and phentolamine mesylate (Ciba-Geigy Japan Ltd., Takarazuka, Japan), clomipramine hydrochloride (Fujiwasa Pharmaceutical Co., Ltd., Osaka, Japan), mianserin hydrochloride and 3-
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quinuclidinyl xanthene-9-carboxylate hemi-oxalate hydrate (Research Biochemicals Inc., Wayland, MA, U.S.A.), cyproheptadine hydrochloride (Merck Sharp & Dohme, Rahway, N.J., U.S.A.), chlorpheniramine maleate (Kowa Co., Ltd., Tokyo, Japan), clemastine fumarate (Sandoz AG, Basel, Switzerland), promethazine hydrochloride and chlorpromazine hydrochloride (Rhône-Poulenc, Paris, France), atropine sulfate (E. Merck AG, Darmstadt, West Germany), quinuclidinyl benzilate and WB4101 (Amersham International), cinanserin hydrochloride (E.R. Squibb & Sons, Princeton, NJ, U.S.A.), spiperone (Eisai Co., Ltd., Tokyo, Japan), haloperidol hydrochloride (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), sulpiride (Teikoku Chemical Ind. Co., Ltd., Osaka, Japan), naloxone hydrochloride (Sanko Co., Ltd., Tokyo, Japan), clonidine hydrochloride (Nippon Boehringer Ingelheim Co., Ltd., Kawanishi, Japan), phenoxybenzamine hydrochloride, propranolol hydrochloride and yohimbine hydrochloride (Nakarai Chemicals, Ltd., Kyoto, Japan) and bicuculline methobromide (Cambridge Research Biochemicals, Ltd., Cambridgeshire, England). Other chemicals of reagent grade were obtained commercially.

Results

Effects of quinupramine on various neurotransmitter receptors: The receptor binding profile is summarized for quinupramine and imipramine (Table 1). Quinupramine was very potent at serotonin S₂, muscarinic cholinergic and histamine H₁ receptors with Kᵢ-values of 2.0, 2.9 and 9.1 nM, respectively. Quinupramine was quite weak at α₁-, α₂- and β-adrenergic, dopamine D₂ receptors and imipramine binding sites.

Saturation of \[^3\text{H}\]\text{Quinupramine} binding: The saturability of \[^3\text{H}\]\text{quinupramine} binding to brain membrane fractions from rat whole brain without cerebellum was examined (Fig. 1). The specific binding of \[^3\text{H}\]\text{quinupramine} was saturable. Scatchard analysis indicated the presence of a single population of binding sites with an equilibrium dissociation constant (Kᵦ) of 3.04±0.05 nM, a maximal binding site number (Bmax) of 714±6 fmol/mg protein and a Hill coefficient (nH) of 1.08±0.02, when non-specific binding was determined in the presence of 10 μM quinupramine. A comparable non-specific binding of \[^3\text{H}\]\text{quinupramine} was observed when non-specific binding was determined by 10 μM mequitazine.

Kinetics of \[^3\text{H}\]\text{Quinupramine} binding: \[^3\text{H}\]\text{Quinupramine} associated rapidly with specific binding attaining half-maximal values at 0.75 min and reaching equilibrium by 4 to 5 min (Fig. 2, upper panel). The association rate constant was calculated on pseudo-first order kinetics using the equation ln[Be/(Be-B)]=Kₒₑ t, where B is the amount of ligand bound specifically at time t and Be is the amount of the specific binding of \[^3\text{H}\]-

| Receptor          | \[^3\text{H}\]Ligand       | Quinupramine | Imipramine |
|-------------------|-----------------------------|--------------|------------|
| α₁- Adrenergic    | 0.22 nM WB4101              | 1,000±58     | 240±40     |
| α₂- Adrenergic    | 0.50 nM Clonidine           | 14,000±710   | 6,300±120  |
| β- Adrenergic     | 0.77 nM Dihydroalprenol     | 48,000±4,800 | 32,000±3,400 |
| Muscarinic cholineric | 0.28 nM Quinuclidinyl benzilate | 2.9±0.2 | 56±4.0     |
| Histamine H₁      | 1.8 nM Pyrilamine           | 9.1±1.1      | 28±1.7     |
| Serotonin S₂      | 0.36 nM Ketanserin          | 2.0±0.4      | 84±17      |
| Dopamine D₂       | 0.23 nM Spiperone           | 2,600±770    | 340±100    |
| Imipramine        | 2.7 nM Imipramine           | 510±22       | 7.9±0.72   |

Standard assay conditions for each of the ligands were used in all experiments. IC50 values (nM) were determined from the log-logit analysis of drug competition data using 6 to 8 concentrations of drugs. Kᵢ values (nM) were calculated from the equation: Kᵢ=IC50/(1+[\[^3\text{H}\]Ligand]/Kₒᵦ). The Kᵢ values were obtained from previous reports (20, 21). Each value represents the mean±S.E.M. of three or more experiments, each performed at least in duplicate.
Fig. 1. Specific binding of \(^{3}H\)quinupramine to rat brain membranes. Membrane preparations were incubated at 37°C for 15 min in 50 mM Tris-HCl buffer (pH 7.7). Non-specific binding was measured by addition of 10 \(\mu M\) quinupramine (●) or 10 \(\mu M\) mequitazine (○). Points shown are the mean±S.E.M. from three independent determinations, each performed in duplicate. Upper panel, total and non-specific binding; lower panel, Scatchard analysis of specific binding.

quinupramine at equilibrium. The observed initial rate constant \((K_{ob})\), the slope of the pseudo-first order plot (Fig. 2, inset of upper panel), was 1.20 min\(^{-1}\). The second order rate constant \((k_1)\) was determined from the equation \(k_1 = (K_{ob} - k_{-1})/\left[ L \right]\), where \([L]\) is the concentration of \(^{3}H\)ligand (1.2 nM). The \(k_1\)-value was 0.273 nM\(^{-1}\) min\(^{-1}\) at 37°C.

The rate of dissociation of \(^{3}H\)quinupramine binding was examined by adding 10 \(\mu M\) quinupramine at equilibrium (Fig. 2, lower panel). The specific binding of \(^{3}H\)quinupramine was reversible. The semilogarithmic plot of the dissociation of \(^{3}H\)quinupramine binding was biphasic. The half-time for dissociation of \(^{3}H\)quinupramine binding was 0.8 min for the initial phase of the curve. The rate constant \((k_{-1})\) for the dissociation of \(^{3}H\)quinupramine binding, determined from the initial dissociation slope, was 0.866 min\(^{-1}\). The kinetically derived \(K_D\)-value for \(^{3}H\)quinupramine binding \((K_D = k_{-1}/k_1)\) was 3.17 nM, and it was very similar to the value obtained in equilibrium experiments \((K_D = 3.04)\).

Effects of various drugs on the specific binding of \(^{3}H\)quinupramine: For 30 compounds belonging to various pharmacological
and chemical classes, IC50 values for inhibition of the specific binding of [3H]-quinupramine to rat brain membrane fractions were determined from inhibition curves as shown in Figs. 3 and 4 (upper panel). The list of compounds, grouped according to their pharmacological activity, and their IC50 values are presented in Table 2. Muscarinic cholinergic antagonists are extremely potent inhibitors of [3H]quinupramine binding, with IC50 values of about 1 nM. Among other drugs, amitriptyline, mequitazine, promethazine and cyproheptadine showed a high to moderate inhibiting potency, and it is well-known that these drugs have substantial affinity for muscarinic cholinergic receptors. By contrast, drugs known to lack antimuscarinic cholinergic activity such as spiperone, WB4101, yohimbine, clonidine, propranolol, diazepam, naloxone and bicuculline were relatively weak inhibitors in [3H]quinupramine bindings.

Differential effects of various drugs on [3H]quinupramine binding in the presence or absence of 10 nM atropine: Inhibition curves of three muscarinic cholinergic antagonists for [3H]quinupramine binding were very similar. Since, about 74% of the [3H]-quinupramine was displaced by these compounds (Fig. 3), the majority of the specific binding of [3H]quinupramine may be attributed to the muscarinic cholinergic receptor binding sites.

Whether or not the binding sites labelled by [3H]quinupramine represent other neurotransmitter receptors was studied using various drugs (Fig. 4). Drug potencies in inhibiting [3H]quinupramine binding in the absence or presence of 10 nM atropine were compared. However, in the absence of atropine, phentolamine, cinanserin and pyrilamine showed about the same affinity for [3H]quinupramine binding with IC50 values of 2,600, 5,000 and 1,500 nM, respectively. Mianserin and cinanserin possessed over 50 times greater affinity for [3H]-quinupramine binding when assays were
Table 2. Inhibition of specific \[^3\text{H}\]quinupramine binding to rat brain membrane fractions by various drugs

| Drugs                  | IC50 (nM) | Drugs                  | IC50 (nM) | Drugs                  | IC50 (nM) |
|------------------------|-----------|------------------------|-----------|------------------------|-----------|
| Antidepressant drugs   |           | Muscarinic cholinergic agents |           | Adrenergic agents      |           |
| Quinupramine           | 4.6±1.1   | Atropine               | 1.1±0.4   | Phentolamine           | 7,600±700 |
| Imipramine             | 190±3     | QNB*                   | 0.99±0.08 | Phenoxybenzamine       | 9,000±2,900 |
| Clomipramine           | 160±20    | QNX*                   | 1.1±0.2   | WB4101                 | 14,000±5,600 |
| Amitriptyline          | 38±5      |                        |           | Yohimbine              | >100,000  |
| Desipramine            | 330±34    | Serotonergic agents    |           | Clonidine              | 83,000±12,000 |
| Mianserin              | 360±60    | Cinanserin             | 5,000±1,200 | Propranolol           | >100,000  |
|                        |           | Cyproheptadine         | 30±1      |                        |           |
| Histaminergic agents   |           | Dopaminergic agents    |           | Chlorpromazine         | 540±79    |
| Mequitazine            | 95±19     |                        |           |                        |           |
| Promethazine           | 29±3      | Spiperone              | 38,000±6,300 | Diazepam              | >100,000  |
| Chlorpheniramine       | 990±80    | Haloperidol            | 3,800±400 | Naloxone               | >100,000  |
| Clemastine             | 4,000±20  | Sulpiride              | >100,000  | Bicuculline            | 81,000±4,200 |
| Pyrilamine             | 1,500±470 |                        |           |                        |           |
| Cimetidine             | >100,000  |                        |           |                        |           |

The inhibition of specific binding of \[^3\text{H}\]quinupramine (1.2 nM) was determined with 5–8 concentrations of competition drugs assayed in duplicate. The mean inhibition concentration values (IC50 in nM) were determined from the log-logit analysis. Values given are the mean±S.E.M. for experiments employing 3–6 separate determinations. *The abbreviations used are: QNB, quinuclidinyl benzilate; QNX, quinuclidinyl xantheine-9-carboxylate.
Table 3. Regional distribution of the specific binding of $[^3H]$quinupramine in rat brain

| Brain region            | $B_{\text{max}}$ (fmol/mg protein) |
|-------------------------|------------------------------------|
| Frontal cortex          | 1,590±106                          |
| Posterior cortex        | 1,270±111                          |
| Striatum                | 1,170±48                           |
| Hippocampus             | 1,170±35                           |
| Hypothalamus            | 843±38                             |
| Midbrain + Thalamus     | 356±70                             |
| Medulla + Pons          | 229±38                             |
| Cerebellum              | 219±17                             |

Specific binding of $[^3H]$quinupramine in each region was determined. $B_{\text{max}}$ values were determined by Scatchard analysis of the binding data. Values given are the mean±S.E.M. of three experiments, each performed in duplicate.

Table 4. Subcellular distribution of the specific binding of $[^3H]$quinupramine in rat whole brain without cerebellum

| Fraction                  | $[^3H]$Quinupramine specifically bound |
|---------------------------|----------------------------------------|
|                           | fmol/mg protein | % of total |
| Standard preparations     | 714±6          |            |
| (50,000×g pellet)         |               |            |
| $P_1$                     | 447±102        | 4.1±0.7    |
| $P_2$                     | 1,050±65       | 73.1±1.8   |
| $P_3$                     | 609±24         | 22.9±1.5   |
| Subfractionations of $P_2$|             |            |
| 0.32 M                    |               |            |
| 0.32–0.8 M (myelin, etc.) | 764±86        | 40.3±5.4   |
| 0.8–1.2 M (synaptosomes)  | 926±25         | 38.0±2.7   |
| 1.2 M (mitochondria)      | 585±99         | 21.8±5.5   |

The specific binding of $[^3H]$quinupramine was determined in various subcellular fractions using the standard assay procedure. The data are presented in terms of specific binding per milligram of protein and as a percentage of the total binding activity recovered. Values given are the mean±S.E.M. of three determinations, each performed in duplicate.

carried out in the presence of atropine, with IC50 values of 2.7 and 98 nM, respectively. By contrast, the affinity of pyrilamine and phentolamine varied less than 7-fold between its affinities for $[^3H]$quinupramine binding in the presence and absence of atropine. The differential effects of these drugs indicated that the binding sites labelled by $[^3H]$quinupramine represented both muscarinic cholinergic and serotonin S2 receptors.

Regional distribution of the specific binding of $[^3H]$quinupramine: The binding in the richest region, frontal cortex, was almost seven times greater than that in the poorest region, cerebellum (Table 3).

Subcellular distribution of the specific binding of $[^3H]$quinupramine: In the principal subcellular fractions of whole homogenates of rat whole brain without cerebellum, the greatest amounts and specific activity of $[^3H]$-quinupramine binding occurred in the crude mitochondrial ($P_2$) fraction (Table 4). When $P_2$-pellets are subfractioned on a discontinuous sucrose gradient designed to separate synaptosomes from mitochondria and myelin as well as other membrane fractions, the greatest specific activity of $[^3H]$quinupramine binding occurs in the synaptosomal fraction.

Discussion

The present study revealed the presence of the specific binding site for $[^3H]$quinupramine in rat brain. Moreover, the subcellular dis-
tribution of high-affinity \(^{3}H\)quinupramine binding and the heterogeneous distribution in brain regions supported the notion that \(^{3}H\)quinupramine attaches specifically to some components of the synaptic membrane.

Scatchard analysis of the specific binding of \(^{3}H\)quinupramine indicated that the binding was saturable (\(B_{\text{max}}=714\) fmol/mg protein), with a single population of binding sites (\(K_D=3.04\) nM). On the other hand, in kinetic studies of \(^{3}H\)quinupramine binding, the dissociation of the binding was biphasic. This discrepancy of the result in Scatchard analysis (single population) and that in the kinetic studies (biphasic dissociation) was also reported on the studies of \(^{3}H\)guanfacine (24) and \(^{3}H\)epinephrine (25) binding. The \(K_D\)-value derived from the equilibrium experiments was very similar to the value derived from the kinetic experiment using the half time for the initial phase of dissociation. Hill analysis of the binding showed no cooperativity (\(n_H=1.08\)).

Many investigators reported the characteristics of the specific binding sites of antidepressant drugs. In all cases, the binding sites had ultimately been found to be neurotransmitter receptors or transport sites. Thus, \(^{3}H\)imipramine (4), \(^{3}H\)norzimelidine (5), \(^{3}H\)Ro 11–2465 (6) and \(^{3}H\)paroxetine (7) bind to a portion of the synaptic transport sites for serotonin; \(^{3}H\)desipramine binds to transport sites of norepinephrine (8); \(^{3}H\)nomifensine binds to transport sites of dopamine (9); \(^{3}H\)mianserin labels both serotonin \(S_2\) and histamine \(H_1\) receptors (10); \(^{3}H\)doxepine labels histamine \(H_1\) receptors (11); \(^{3}H\)tetrahydrotrazodone labels serotonin \(S_2\) receptors (12); and \(^{3}H\)amitriptyline labels multiple receptor sites (13). It is conceivable that the specific binding sites of \(^{3}H\)quinupramine are central serotonin \(S_2\), muscarinic cholinergic and/or histamine \(H_1\) type receptors, because quinupramine was found to possess high affinity for central serotonin \(S_2\), muscarinic cholinergic and histamine \(H_1\) receptors. To examine this point, the pharmacological characteristics of the specific binding sites of \(^{3}H\)quinupramine were studied using different agents. The results indicated that the binding of \(^{3}H\)quinupramine can be accounted for by both muscarinic cholinergic and serotonin \(S_2\) receptors. In the central nervous system, muscarinic cholinergic systems may predominate over serotonergic systems, since the density of muscarinic cholinergic receptor (\(B_{\text{max}}=981\) fmol/mg protein, assessed by \(^{3}H\)quinuclidinyl benzilate binding in whole brain without cerebellum) is higher than that of serotonin \(S_2\) receptors (\(B_{\text{max}}=243\) fmol/mg protein, assessed by \(^{3}H\)ketanserin binding in rat frontal cortex) (18).

In general, tricyclic antidepressant drugs are fairly potent inhibitors for agonists at muscarinic cholinergic, histamine \(H_1\) and serotonin \(S_2\) receptors, and these anti-muscarinic cholinergic and anti-histaminergic actions are thought to correlate well with clinical side effects, i.e., dry mouth, mydriasis and sedation (26). On the other hand, the anti-serotonergic action is thought to correlate with the anti-depressant activity (27). However, it is unlikely that the acute blockade of central neurotransmitter binding sites relates to antidepressant activity (26), and it is likely that antidepressant drugs may not have a common mechanism of action. Quinupramine may affect various neurotransmitter systems, which play important roles in the etiology of affective disorders through muscarinic cholinergic and/or serotonergic systems, but further studies are required to clarify the role of central muscarinic cholinergic and serotonergic receptors in the antidepressant efficacy of quinupramine.

Mequitazine is a potent antihistamic drug with a potent antimuscarinic cholinergic activity (28), and it has a quinuclidine group as a moiety in the molecule. Though the chemical structure of mequitazine is very close to that of quinupramine, mequitazine has weak antiserotonergic activity (29) and no antidepressant activity.

If the specific binding of \(^{3}H\)quinupramine involves central neurotransmitter receptor binding sites, this binding should be distributed unevenly in the brain. The cerebral cortex had the highest regional distribution of \(^{3}H\)quinupramine specific binding, while the cerebellum had the lowest. The highest specific binding of \(^{3}H\)ketanserin, a ligand for serotonin \(S_2\) receptors, was determined in
the frontal cortex in rat brain, and specific binding was virtually not detected in the hypothalamus and cerebellum (30). Similarly, the highest specific binding of \([3H]\)quinuclidinyl benzilate, a ligand for muscarinic cholinergic receptors, was detected in the striatum and the lowest in the cerebellum (31). The data of the regional distribution of \([3H]\)quinupramine binding, which labels both serotonin S\(_2\) and muscarinic cholinergic receptors, may be not contrary to these observations.

Neurotransmitter receptors are generally assumed to involve postsynaptic membranes. The specific binding of \([3H]\)quinupramine in the various subcellular fractions prepared by centrifugation techniques was greatest in fractions containing high concentrations of synaptic membranes and synaptosomes. This suggests the \([3H]\)quinupramine binds predominantly to synaptic receptor sites, although some binding to non-neural systems was not excluded.

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