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Biochemical Characterization of the Muscarinic Cholinergic Receptor in Human Brain: Alterations in Huntington's Disease

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SUMMARY

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[^{3}H]3-Quinuclidinyl benzilate was used to characterize the muscarinic cholinergic receptor binding in three regions of the normal human brain and in rat brain. In each of these tissues, specific ligand binding was linear between 0.05 and 0.7 mg tissue protein/assay at [^{3}H]QNB concentrations between 20 and 200 pM. For the human brain regions, the bimolecular rate constant of association was 6.9×10^8 M^{-1} min^{-1} and the dissociation rate constant was 14.3×10^{-3} min^{-1}. The K_D calculated from the ratio of these two values was 20.1 pM. The kinetic K_D determined for rat brain, under these same conditions, was 25 pM. The dissociation rate constant and the kinetic K_D for both the human brain regions and the rat brain were similar whether dissociation was initiated before or after the ligand-receptor interaction had achieved equilibrium.

Scatchard analysis of saturation isotherms, using similar receptor concentrations for each of the human brain regions, gave K_D values of 60–70 pM with binding capacities between 600 and 900 fmol/mg protein. Analysis of saturation data from rat brain using similar receptor concentrations gave a K_D of 70 pM and a binding capacity of 900 fmol/mg protein.

[^{3}H]Quinuclidinyl benzilate binding was inhibited by muscarinic agonists and antagonists; the antagonists were 1000-fold more potent than were the agonists. Under the specified conditions the antagonists and agonists had IC_{50} values between 0.1–2 nM and 0.8–10 μM, respectively. The muscarinic antagonists had Hill coefficients of 1.0 and the agonists had Hill coefficients between 0.70 and 0.85. Nicotinic and noncholinergic drugs were ineffective in inhibiting [^{3}H]quinuclidinyl benzilate binding at concentrations up to 10 μM.

Huntington's diseased brains had significant decreases in muscarinic receptor binding in the caudate nucleus and putamen. Choline acetyltransferase activity was decreased significantly in the caudate nucleus, putamen and globus pallidus of Huntington's diseased brains.

Saturation isotherms for [^{3}H]quinuclidinyl benzilate binding were determined in each of the three control brain regions and in Huntington's diseased putamen at different

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receptor concentrations. Each of these brain regions had a "true" K_D between 20 and 40 pM. These studies, plus inhibition studies using dexetimide, the biologically-active stereoisomer of benzetimide, revealed that the decreases in ligand binding were due to receptor loss rather than to changes in receptor affinity.

INTRODUCTION

The reversible muscarinic antagonist [^{3}H]QNB[3] was used originally to characterize the muscarinic cholinergic receptor in rat brain (1) and in the longitudinal smooth muscle of the guinea-pig ileum (2). Using both autoradiographical methods and *in vitro* binding studies, [^{3}H]QNB has since proven useful in determining the number and regional distribution of muscarinic receptors in monkey brain (3) and in normal and HD human brains (4).

It has been shown that there are decreases in muscarinic receptor binding in the corpus striatum of HD brains (4–6). To date, however, a detailed biochemical characterization of the muscarinic receptors in normal human brain, as compared with those in the choreic brain, has not been done.

The purposes of the present study were to do such a detailed biochemical characterization of the muscarinic receptor in human brain, to compare these data with those from rat brain, and to compare the control data with those from HD brains in order to establish the nature of the muscarinic receptor alterations involved in HD.

MATERIALS AND METHODS

Pathological specimens of human brains were collected from various hospitals throughout the United States. The brains were divided into two groups: control brains and those from patients diagnosed as having HD. Cases with infectious or malignant diseases of the central nervous system were eliminated from the control group. Clinical case histories, including lists of prescribed medication(s), were obtained for each patient. None of the patients in either group had been treated with cholinergic drugs prior to his time of death. The maximum delay between time of death and autopsy was 24 hours. Immediately after autopsy, the brains were frozen in dry ice for transport to our laboratory. Upon arrival, they were dissected and prepared for analysis. Twenty-six brain regions were used for this study.

The brain regions were homogenized, using a Polytron homogenizer (setting #5 for 30 sec), to either a 3% or a 1% homogenate (w/v) in 0.05 M phosphate buffer (81 mM Na^+, 9 mM K^+, pH 7.4). An aliquot (0.1 ml) of the unwashed homogenate was saved for analysis of ChAc activity. Additional 0.1 ml aliquots were analyzed for protein by the method of Lowry *et al.* (7). The remainder of each homogenate was then washed by diluting it with 15 ml of phosphate buffer and centrifuging it at 48,000 × *g* for 15 min in a Sorvall RC2-B centrifuge. The supernatant was discarded and the pellet was resuspended in the appropriate volume of phosphate buffer. For the standard binding assay, aliquots (0.05 ml or 0.1 ml of a 3% or 1% homogenate, respectively) of the washed suspension were added to two sets of triplicate culture tubes; one set (total binding) contained 200 pM [^{3}H]QNB in 2 ml phosphate buffer. The other set (nonspecific binding) contained 200 pM [^{3}H]-QNB and 0.01 μM unlabelled atropine in 2 ml phosphate buffer. Both sets of tubes were routinely incubated at 37°C for 60 min. The suspensions were then filtered through glass-fiber filters (Whatman GF/B). Each of the culture tubes was rinsed twice with 5 ml of ice-cold buffer which was then poured over the appropriate filter. All filters were washed an additional two times with 5 ml of ice-cold buffer. The filters were placed into liquid scintillation vials containing 6 ml Triton-toluene Omnifluor (1 liter:2 liter:16 g) liquid scintillation cocktail. The filtered tissue was allowed to extract for twelve hours and was then counted in a Nuclear-Chicago Isocap/300 liquid scintillation spectrometer

[3] Abbreviations used are: [^{3}H]QNB, [^{3}H]3-quinuclidinyl benzilate; HD, Huntington's disease; ChAc, choline acetyltransferase.

at a counting efficiency of 45%. Specific [^{3}H]QNB binding for each region, expressed as fmol [^{3}H]QNB/mg protein, was calculated by subtracting the nonspecific from the total binding.

Studies involving the inhibition of [^{3}H]-QNB binding by various drugs were performed in two sets of triplicate culture tubes; one set contained 10 ml of phosphate buffer, 0.05 ml of the 3% suspension or 0.1 ml of the 1% suspension, and 39 pM [^{3}H]-QNB. The other set contained, in addition, an unlabelled drug. All drugs were obtained from commercial suppliers. Dexetimide and levetimide were provided by Elliot Richelson (Mayo Clinic, Rochester, Minn.). Unlabelled QNB was furnished by the Roche Co.

To determine the dissociation rate of [^{3}H]QNB from the muscarinic receptor two sets of triplicate culture tubes, containing tissue and buffer, were equilibrated with [^{3}H]QNB for 60 min at 37°C. At 60 min, unlabelled atropine (1 μM) was added to one set of the tubes. At various time intervals, thereafter, the tissue was filtered through GF/B filters. In an alternate method for determining the dissociation rate, portions of a single batch of the [^{3}H]-QNB-receptor complex were divided among several flasks and dissociation was initiated by addition of either unlabelled atropine, unlabelled QNB or by dilution. The dissociation rate constants derived by each of these methods were then compared (8).

ChAc activity was measured in the unwashed homogenate by a modification of the method of Yamamura et al (9). ChAc activity was expressed as (nmol ACh synthesized) (mg protein)$^{-1}$ (hr)$^{-1}$.

Radiolabelled QNB was prepared by base-catalyzed ester exchange between methyl benzilate and [3-^{3}H]quinuclidinol at New England Nuclear, Boston, Mass. The purified compound had a specific activity of 13 Ci/mmol. Radiolabelled acetyl-CoA was purchased from New England Nuclear at a specific activity of 60 mCi/mmol.

ANALYSIS OF BINDING DATA

Analysis of the interaction between [^{3}H]QNB and the muscarinic receptor was made on the basis of the following model

$$D + R \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} DR \tag{1}$$

where D = [^{3}H]QNB, R = the muscarinic receptor and DR = the [^{3}H]QNB-receptor complex.

The rate constant for formation of the [^{3}H]QNB-receptor complex was determined from the second-order equation:

$$\ln\left[\frac{(DR)_{eq}\cdot\left[D_T - DR\cdot\dfrac{(DR)_{eq}}{R_T}\right]}{D_T\cdot[(DR)_{eq} - (DR)]}\right] = k_{+1}\cdot t\left[\left[D_T\cdot\frac{R_T}{(DR)_{eq}}\right] - (DR)_{eq}\right] \tag{2}$$

$(DR)_{eq}$ = the equilibrium concentration of the [^{3}H]QNB-receptor complex, D_T = the initial free [^{3}H]QNB concentration and DR = the concentration of the [^{3}H]QNB-receptor complex at time t. The $t½_{assoc}$ values were determined by the method of Maelicke *et al.* (10).

The rate constant for dissociation of the [^{3}H]QNB-receptor complex was determined from the equation:

$$k_{-1} = 0.693/t_{1/2} \tag{3}$$

where $t½$ = the amount of time necessary for one-half of the [^{3}H]QNB-receptor complex to dissociate. The K_D can then be calculated from the ratio of the rate constants:

$$K_D = k_{-1}/k_{+1} \tag{4}$$

Data from studies involving inhibition of [^{3}H]QNB binding by various drugs were represented as percent of control. From Hill (11) plots, IC_{50} values and Hill coefficients were calculated to determine the relative binding potencies of these drugs for the muscarinic receptor, and to determine whether the interaction of these drugs with the muscarinic receptor adheres to mass-action kinetics. Saturation isotherms were analyzed according to the method of Scatchard (12, 13).

In systems where a high-affinity ($K_D \leq 10^{-10}$ M) ligand is used, it may be difficult to make a direct estimate of the "true" K_D

from the total ligand concentration at which 50% of the receptors are occupied. A derivation of the equation, $K_D = (D)(R)/(DR)$ (see Eq. 1), done according to the method of Chang, Jacobs and Cuatrecasas (14), makes this immediately apparent:

$$D_T = K_D (f/1 - f) + f(R_T) \quad (5)$$

Equation (5) shows the relationship between the fractional receptor occupancy, f, the ligand concentration, D_T, and the receptor concentration, R_T. At one extreme, if $R_T < 0.1\ K_D$, then Eq. (5) becomes $D_T \cong K_D (f/1 - f)$ where the K_D is essentially independent of the receptor concentration. Under these circumstances, the ligand concentration at which half-maximal binding occurs approximates the true K_D.

At the other extreme, if $R_T > 100\ K_D$, however, Eq. (5) becomes $D_T \cong f(R_T)$ and the free ligand concentration, hence the K_D, becomes dependent upon the receptor concentration. Therefore, the ligand concentration at which half-maximal binding occurs approximates the true K_D only if the receptor concentration is less than one-tenth of the true K_D. In very high-affinity systems, as with [^{3}H]QNB binding to the muscarinic receptor, such conditions may be difficult to approach experimentally. It was for this reason that we titrated the K_D apparent ($K_{D\ app}$) versus the receptor concentration, among the control and HD regions, to see if their receptor affinities were unaltered as well as to establish a true K_D for the normal muscarinic receptor.

RESULTS

Of the 26 human brain regions studied, three regions of the control human brains (the putamen, the frontal cortex and the hippocampus) were used to study the affinity of [^{3}H]QNB binding for the muscarinic receptor. Specific [^{3}H]QNB binding was linear between 0.05 and 0.7 mg tissue protein/assay at [^{3}H]QNB concentrations between 20 and 200 pM (Fig. 1). Specific [^{3}H]QNB binding in rat brain was linear within the same range of tissue protein values.

[^{3}H]QNB binding is a time-dependent process requiring 40–50 min to reach equilibrium (Fig. 2) depending upon the ligand and tissue concentrations used. At 37°C, 88 pM [^{3}H]QNB and a tissue protein concentration of 0.05 mg/assay, specific [^{3}H]QNB binding reached equilibrium by 50 min in the three regions of the human brain and in the rat brain. When the data were ana-

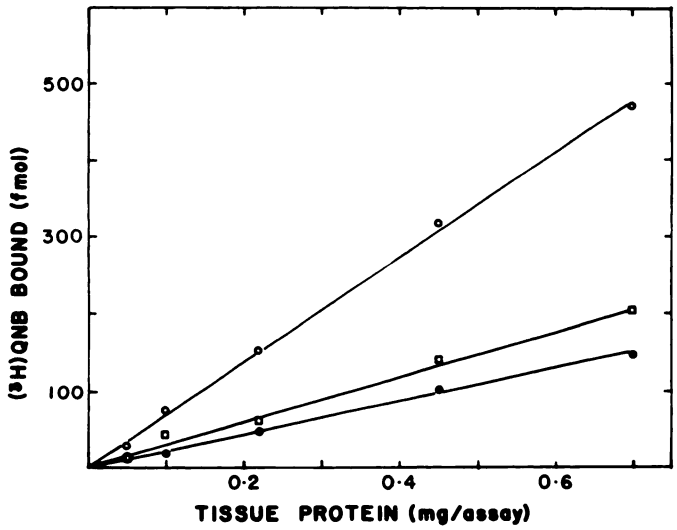

FIG. 1. *Specific binding of [^{3}H]QNB to the muscarinic receptor in three human brain regions [○ putamen; □ frontal cortex; ● hippocampus] as a function of tissue protein concentration*

Unlabelled atropine 0.1 μM was used as the displacer of specific [^{3}H]QNB binding. Tissue linearity existed for all [^{3}H]QNB concentrations between 20 and 200 pM [^{3}H]QNB.

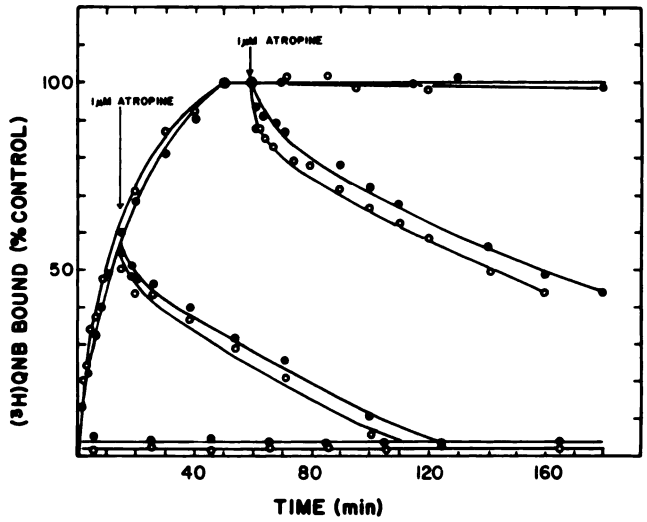

FIG. 2. *Association and dissociation of specific [³H]QNB binding to the muscarinic receptor in the human putamen* [●] *and in rat brain* [○] *as a function of time*

The assay was performed at 37°C using 88 pM [³H]QNB and a receptor concentration of 10 pM. The bottom two lines represent non-specific [³H]QNB binding.

lyzed according to the method of Maelicke *et al.* (10), the $t½_{assoc}$ values for both the human putamen and whole rat brain were approximately 10 min at the specified tissue and ligand concentrations (Fig. 3). The other human brain regions also had $t½_{assoc}$ values of approximately 10 min under these same conditions (data not shown).

The half-time for dissociation in all three human brain regions (100–110 min) was slower than that for rat brain (70–80 min) (Fig. 4). The bimolecular rate constant of association was $6.9 \times 10^8 M^{-1}$ min^{-1} and the dissociation rate constant was 14.3×10^{-3} min^{-1} in the human putamen. The K_D calculated from the ratio of these two values was 20.1 pM. The kinetic K_D determined for rat brain, under the same experimental conditions, was 25 pM. The dissociation rate constant and the kinetic K_D for both the human brain regions and the rat brain were similar whether dissociation was initiated before or after the ligand-receptor interaction had achieved equilibrium (Fig. 2). The dissociation rate constant was similar ($\approx 14.4 \times 10^{-3}$ min^{-1}) when the volume of buffer was increased 100-fold to initiate the dissociation of the bound [³H]QNB.

Saturation of the muscarinic receptor by [³H]QNB was measured at [³H]QNB concentrations between 9 and 260 pM and at receptor concentrations between 25 and 30 pM for each of the three human brain regions. Under these conditions, specific [³H]QNB binding was saturable in all three brain regions whereas nonspecific binding was not. Specific [³H]QNB binding was approximately 90% of the total binding at 200–260 pM and 95% of the total binding at 9 pM [³H]QNB (Fig. 5). Scatchard analysis of these saturation isotherms gave K_D values for all three regions between 60–70 pM with binding capacities between 600 and 900 fmol/mg protein (Fig. 6). Scatchard analysis of the saturation isotherms from five additional human brain regions (the parietal cortex, the amygdala, the hypothalamus, the substantia nigra and the pulvinar of the thalamus) also gave K_D values between 60–80 pM (data not shown). Analysis of saturation data from rat brain, using a similar receptor concentration, gave a K_D of 70 pM and a binding capacity of 900 fmol/mg protein. Since there was no significant difference among the regional K_D values, one concentration of [³H]QNB was used in subsequent examinations of the regional distribution of the muscarinic receptor in human brain.

Muscarinic agonists and antagonists ef-

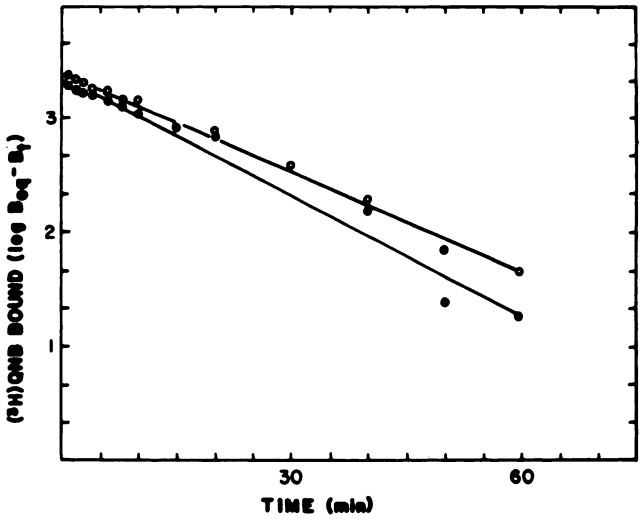

FIG. 3. *Rate of association of specific [^{3}H]QNB binding to the muscarinic receptor in the human putamen* [●] *and in rat brain* [○] *plotted according to the method of Maelicke et al (10)*

$t½_{assoc}$ = 10 min for both tissues; r = 0.99. The ordinate represents the log of the difference between the amount of the [^{3}H]QNB bound specifically at equilibrium (60 min) and that bound at time "t".

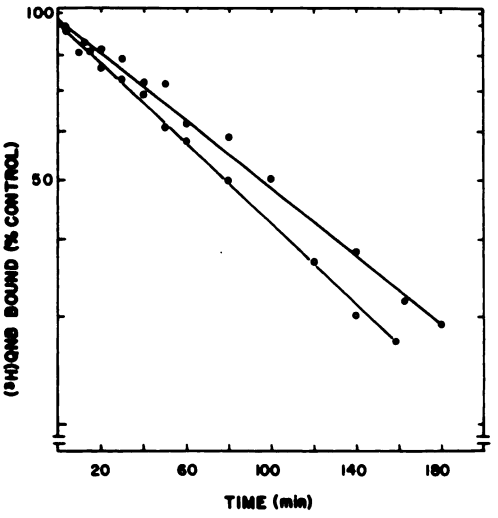

FIG. 4. *Rate of dissociation of specific [^{3}H]QNB binding from the muscarinic receptor in the human putamen* [●] *and in rat brain* [○] *plotted semilogarithmically, as % control, with time*

$t½_{diss}$ for putamen = 100 min; $t½_{diss}$ for rat brain = 80 min. r = 0.99 for both lines.

fectively inhibited [^{3}H]QNB binding in human brain (Fig. 7). The antagonists were approximately 1000-fold more potent as inhibitors of [^{3}H]QNB binding than were the agonists (Fig. 8). Dexetimide, the D-stereoisomer of the muscarinic antagonist benzetimide, was approximately 1000-times more potent in inhibiting [^{3}H]QNB binding than was the L-stereoisomer, levetimide. The relative potencies of the various inhibitors, given as their IC_{50} values in the human frontal cortex, and their Hill coefficients are shown in Table 1. Nicotinic and noncholinergic drugs were ineffective in inhibiting [^{3}H]QNB binding at concentrations up to 10 μM. These drugs were: d-tubocurare, hexamethonium, nicotine, norepinephrine, serotonin, diazepam, glutamate, taurine, gamma-aminobutyric acid (GABA) and muscimol.

Specific [^{3}H]QNB binding is highest in the telencephalon and lowest in the brain stem of the control brains. There were significant decreases in [^{3}H]QNB binding in the caudate nucleus and putamen of HD brains (Table 2). [^{3}H]QNB binding in the putamen decreased from approximately 700 fmol/mg protein to 250 fmol/mg protein in the HD brains. Binding in the caudate nucleus decreased from approximately 950 fmol/mg protein to 300 fmol/mg protein. There were also significant decreases in ChAc activity in the caudate nucleus, the putamen and the globus pallidus of HD brains (Table 3).

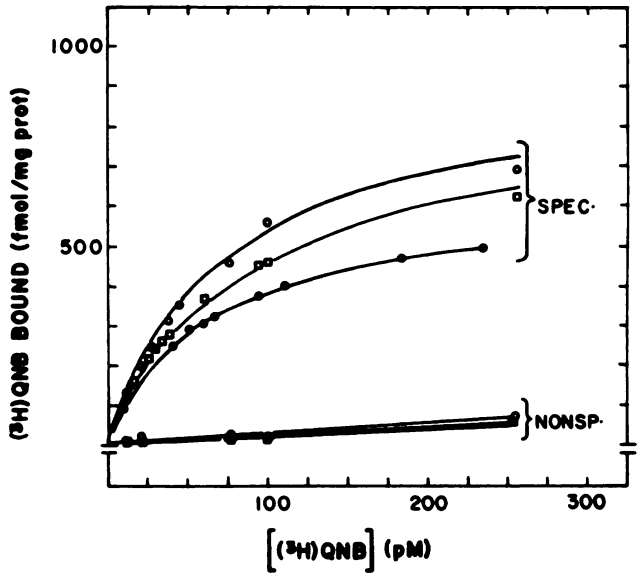

FIG. 5. *Specific binding of [^{3}H]QNB to the muscarinic receptor in three human brain regions [○ putamen; □ frontal cortex; ● hippocampus] as a function of the [^{3}H]QNB concentration*
Receptor concentrations between 25 and 30 pM were used for each of the three brain regions.

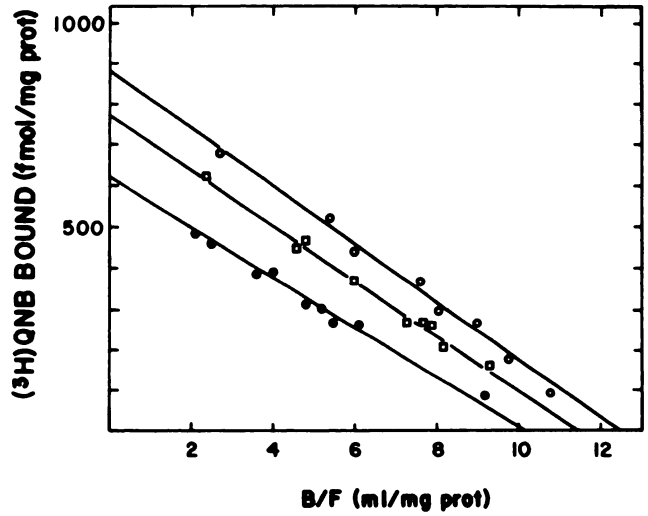

FIG. 6. *Scatchard plot of the saturation isotherms shown in Fig. 5*
K_D values = 60–70 pM. Binding capacities = 600–900 fmol/mg protein. r values = 0.97–0.99.

When dexetimide, the biologically-active stereoisomer of benzetimide, was used to inhibit [^{3}H]QNB binding in the HD putamen, the curve generated (when expressed as % control) showed no significant difference in slope or extent from that of the control putamen (Fig. 9). However, the same curve, when expressed as [^{3}H]QNB bound (fmol/mg protein) showed that specific binding in the HD putamen was less than 50% of that in control tissue (Fig. 9).

Saturation isotherms for [^{3}H]QNB binding were determined in each of the three control brain regions at different receptor concentrations. The K_D values derived from Scatchard analysis of these saturation data

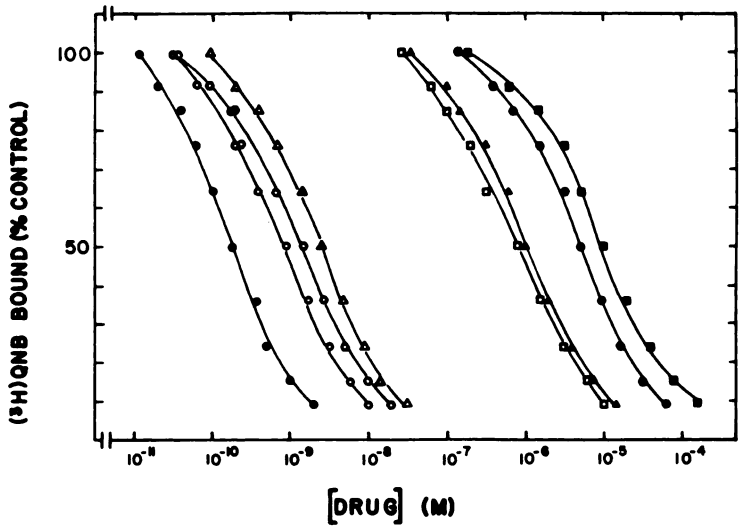

FIG. 7. *Inhibition of [³H]QNB binding to the muscarinic receptor in human frontal cortex by muscarinic agonists [□ oxotremorine; ● pilocarpine; ■ acetylcholine] and antagonists [● QNB; ○ scopolamine; ⬡ atropine; △ dexetimide; ▲ levetimide].*

39 pM [³H]QNB was incubated with a receptor concentration of approximately 15 pM.

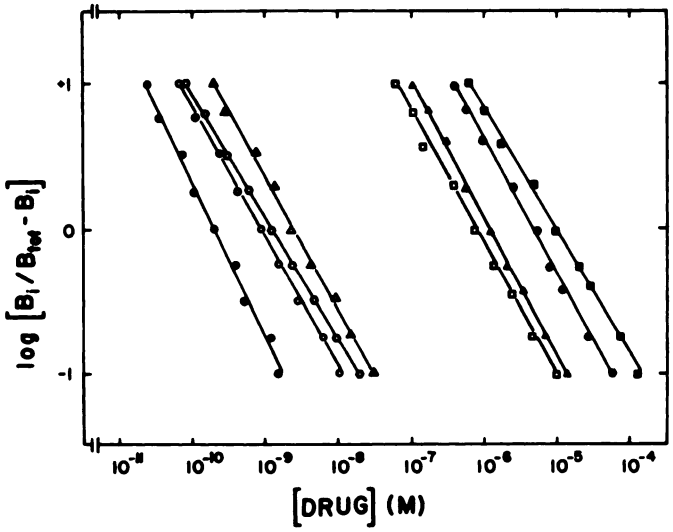

FIG. 8. *Hill plot of the inhibition data shown in Fig. 7*

B_i = [³H]QNB bound in the presence of a given concentration of inhibitor. B_{tot} = [³H]QNB bound in absence of inhibitor. Hill coefficients equal the negative slope of the inhibition line.

were plotted versus receptor concentration for each control region (Fig. 10). By extrapolating the linear regression line to the ordinate (i.e., to an infinitely small receptor concentration) one can determine the true K_D for that region. Each of the control brain regions had a true K_D between 20 and 40 pM. A similar analysis was done using data from choreic putamen and the true K_D was not significantly different from those of the normal brain regions (Fig. 10).

DISCUSSION

The main conclusions of this study are

TABLE 1

Inhibition of [^{3}H]QNB binding to the muscarinic receptor in the human frontal cortex by muscarinic agonists and antagonists

IC_{50} values and Hill coefficients for muscarinic agonists and antagonists as determined from the data in Fig. 8. Nicotinic and noncholinergic drugs ineffective in inhibiting [^{3}H]QNB binding at concentrations up to 10 μM: d-tubocurare, hexamethonium, nicotine, norepinephrine, serotonin, diazepam, glutamate, taurine, GABA and muscimol.

Inhibitor	IC_{50}	Hill coefficient
	(nM)	
QNB	0.1	1.00
Scopolamine	0.8	1.00
Atropine	1	0.98
Dexetimide	2	0.99
Oxotremorine	800	0.85
Levetimide	1,000	0.75
Pilocarpine	5,000	0.71
Acetylcholine"	10,000	0.70

" Physostigmine (1 μM) was used to prevent hydrolysis of ACh.

that the binding characteristics of the muscarinic cholinergic receptor in human brain and in rat brain are similar, and that the alterations in muscarinic receptor binding in Huntington's disease are due to a decrease in the number of receptors rather than to a change in their affinity. The value of the present study is that it combines a detailed characterization of both normal and choreic muscarinic receptors with a thorough discussion of the analytical methods used to assess receptor binding data.

We felt it necessary to satisfy three criteria in selecting the tissues to be used for characterizing the human brain muscarinic receptor: (1) the binding in these tissues should be high enough to make any binding alterations readily apparent; (2) it should be possible to differentiate the tissues on the basis of their binding capacities alone; and (3) the tissues should be from different functional systems (e.g., the limbic system, etc.) so that binding alterations in any one of them would suggest functional correlates for that alteration. After screening twenty-six human brain regions, we chose the putamen, the frontal cortex and the hippocampus to fulfill these criteria. The homogenates of these regions were washed by dilution and centrifugation to remove any medication(s) or endogenous substances which might interfere with the [^{3}H]QNB binding assay.

Specific [^{3}H]QNB binding increased linearly with increasing tissue protein concentrations. A tissue protein concentration of 0.2 mg/assay or less was used routinely to ensure that specific binding was as far below 10% of the free [^{3}H]QNB concentration as possible (see ANALYSIS OF BINDING DATA).

Kinetic calculations, using Eq. (2), showed that the k_{+1} for [^{3}H]QNB binding is the same in all three human brain regions and in the rat brain.

The rate constants for dissociation of the [^{3}H]QNB receptor complex, under the specified conditions, are similar for the three brain regions. However, the dissociation rate constants for the three regions are less

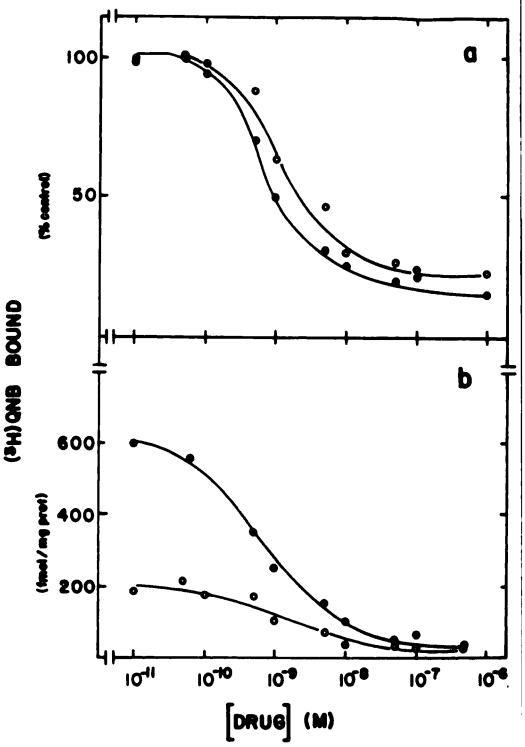

FIG. 9. *Inhibition of [^{3}H]QNB binding to the muscarinic receptor in control* [●] *and HD putamen* [○] *by dexetimide*

Expressed as: (a) % control and (b) fmol/mg tissue protein. 20 pM [^{3}H]QNB was incubated with approximately 25 pM receptor of both normal and HD putamen.

TABLE 2

Specific [^{3}H]QNB binding in various regions of control and HD brain

Region	[^{3}H]QNB binding in human brain[d]	
	Control	HD
	(fmoles/mg tissue protein)	
Caudate Nucleus	(6)[a] 949.67 ± 44.08[c]	(5) 314.00 ± 68.76[b]
Putamen	(9) 706.78 ± 95.83	(6) 250.83 ± 19.98[b]
Parietal Cortex	(3) 559.67 ± 103.57	(4) 532.25 ± 90.36
Occipital (Calcarine) Cortex	(8) 540.25 ± 85.51	(5) 540.80 ± 112.56
Frontal Cortex	(9) 520.55 ± 89.19	(5) 624.60 ± 81.21
Amygdala	(7) 471.00 ± 105.15	(6) 479.83 ± 60.03
Precentral Gyrus	(4) 389.75 ± 73.97	(5) 388.50 ± 101.83
Postcentral Gyrus	(4) 386.00 ± 84.12	(5) 400.00 ± 101.31
Hippocampus	(9) 319.89 ± 59.43	(6) 305.17 ± 50.11
Pulvinar of Thalamus	(2) 219.00 ± 60.00	(2) 197.50 ± 40.5
Tectum of Midbrain	(3) 141.67 ± 21.17	(3) 229.67 ± 152.32
Hypothalamus	(3) 129.00 ± 35.51	(3) 61.67 ± 28.90
Globus Pallidus	(9) 124.89 ± 47.63	(5) 69.20 ± 13.91
Basis Pons	(7) 85.43 ± 33.57	(6) 114.17 ± 36.23
Tegmentum of Medulla	(6) 68.50 ± 25.54	(3) 31.33 ± 18.80
Mammillary Body	(6) 60.33 ± 13.87	(3) 37.00 ± 17.06
Substantia Nigra	(9) 46.33 ± 16.43	(4) 43.75 ± 16.45
Red Nucleus	(6) 38.33 ± 12.98	(3) 16.33 ± 16.33
Inferior Olivary Nucleus	(9) 20.55 ± 8.14	(4) 36.00 ± 30.30
Basis Medulla	(3) 20.33 ± 13.17	(2) 20.50 ± 20.50
Interpeduncular Area	(7) 20.14 ± 13.96	(3) 42.00 ± 15.14
Corpus Callosum	(7) 15.86 ± 8.11	(4) 36.75 ± 10.82
Cerebellar Hemisphere	(7) 14.71 ± 9.50	(4) 22.75 ± 13.70
Posterior Cerebellar Vermis	(8) 13.00 ± 6.71	(5) 30.40 ± 13.16
Dentate Nucleus	(6) 12.17 ± 7.78	(6) 20.83 ± 10.73
Anterior Cerebellar Vermis	(8) 9.50 ± 5.44	(6) 18.50 ± 9.40

[a] Number in parenthesis = n.

[b] Significant to a level of $p < 0.01$.

[c] Mean ± SEM.

[d] [^{3}H]QNB (200 pM) was incubated with a receptor concentration of ≈20 pM using 0.01 μM atropine as the displacer.

than those for rat brain, suggesting that there may be some subtle difference(s) between muscarinic receptors in human and rat brain. However, the kinetic K_D values for both the human brain regions and the rat brain are similar. This difference does not appear to be due to desensitization of the receptor with time (15); if the dissociation reaction is initiated at 15 min instead of 60 min (i.e., before the formation of the [^{3}H]QNB-receptor complex has reached equilibrium) the human brain regions still have a slower dissociation rate than does the rat brain, and the k_{-1} values remain the same.

The fact that muscarinic agonists and antagonists inhibit [^{3}H]QNB binding at concentrations paralleling their pharmacological potency (1, 2), and the fact that nicotinic and noncholinergic drugs inhibit binding only slightly at 10 μM concentrations, indicate that [^{3}H]QNB is interacting with the human brain muscarinic receptor. In addition, the extent of the inhibition of [^{3}H]QNB binding is similar for all the muscarinic drugs tested indicating that these drugs are interacting with the same receptor population (16).

The antagonists had Hill coefficients of 1.0 and the agonists had Hill coefficients of less than 1.0. Because of this deviation from mass-action kinetics (16, 17), even at the low tissue and ligand concentrations used, the IC_{50}s for the muscarinic agonists cannot be considered to reflect the thermodynamic inhibitory constant (K_i) values. These low

TABLE 3

Choline acetyltransferase (ChAc) activity in various regions of control and HD brains

Region	ChAc activity in human brain[e] Control	HD
	[(nmoles Ach) (mg tissue protein)$^{-1}$ (hr)$^{-1}$]	
Putamen	(5)[a] 42.33 ± 6.38[d]	(6) 16.34 ± 11.09[c]
Caudate Nucleus	(5) 38.99 ± 7.34	(5) 12.26 ± 4.36[b]
Globus Pallidus	(5) 13.43 ± 3.14	(5) 4.52 ± 1.18[c]
Dentate Nucleus	(3) 8.23 ± 5.02	(6) 2.57 ± 0.67
Amygdala	(4) 7.29 ± 2.11	(6) 8.96 ± 4.29
Hypothalamus	(5) 5.18 ± 2.01	(4) 3.52 ± 0.83
Posterior Cerebellar Vermis	(5) 5.10 ± 2.08	(5) 2.40 ± 0.55
Tegmentum of Pons	(5) 4.84 ± 0.79	(4) 6.41 ± 1.82
Anterior Cerebellar Vermis	(6) 4.70 ± 1.46	(6) 2.89 ± 0.71
Temporal Cortex	(3) 4.61 ± 1.45	(3) 3.90 ± 0.90
Hippocampus	(5) 3.99 ± 1.12	(6) 3.04 ± 1.04
Thalamus	(5) 3.93 ± 0.90	(4) 2.61 ± 0.41
Calcarine Cortex	(3) 3.37 ± 0.87	(3) 2.28 ± 0.56
Paprietal Cortex	(3) 3.29 ± 0.91	(3) 2.59 ± 0.54
Basis of Pons	(5) 3.18 ± 0.89	(6) 2.86 ± 0.79
Occipital Cortex	(3) 3.10 ± 1.14	(3) 1.93 ± 0.42
Red Nucleus	(4) 2.81 ± 0.53	(3) 3.06 ± 1.20
Inferior Olivary Nucleus	(6) 2.79 ± 0.38	(3) 3.76 ± 1.86
Substantia Nigra	(5) 2.57 ± 0.52	(4) 3.22 ± 1.38
Frontal Cortex	(5) 2.23 ± 0.55	(4) 3.24 ± 0.75
Cerebellar Hemisphere	(5) 1.78 ± 0.32	(4) 1.50 ± 0.40

[a] Number in parenthesis = n.

[b] Significant to a level of $p < 0.01$.

[c] Significant to a level of $p < 0.05$.

[d] Mean ± SEM.

[e] 0.005 ml of a 3% homogenate (w/v) from each brain region was incubated at 37°C for 20 min with 0.025 ml of ChAc cocktail (see METHODS section). The reaction was terminated by the addition of 0.1 ml tetraphenylboron in 3-heptanone (50 mg/ml).

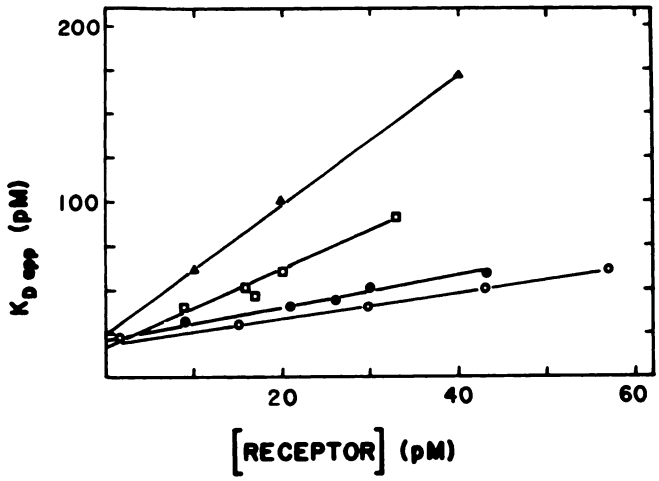

FIG. 10. *Relationship between the apparent dissociation constant, $K_{D_{app}}$, and the receptor concentration in HD putamen* [▲], *frontal cortex* [□], *hippocampus* [●] *and putamen* [○]

Each point on this graph represents the $K_{D_{app}}$, as determined by Scatchard analysis of saturation data, for each brain region at the specified receptor concentration.

Hill coefficients may be explained in the following ways: (1) the muscarinic receptor is progressively desensitized by exposure to increasing agonist concentrations (18); (2) there is more than one population of muscarinic receptors, each having a different affinity for the agonist (19); or (3) there is negative cooperativity between the agonist and antagonist conformations of the receptor (20). Since most of the radioactive ligands used to date to study the muscarinic receptor have been antagonists (1-2, 18, 19, 21-27), and presumably bind to the antagonist population or conformation of the receptor, the use of a radiolabelled muscarinic agonist in binding assays should clarify this situation.

Scatchard analysis of the saturation isotherms in each of the three control brain regions indicated no significant differences among the K_D values determined for each region. The K_D values derived from saturation studies are higher than those determined from kinetic measurements. The kinetic measurements, however, better reflect the true K_D because the calculations of the association rate constant (k_{+1}) and the dissociation rate constant (k_{-1}), hence the kinetic K_D, are less dependent upon the receptor and ligand concentrations than is Scatchard analysis of saturation isotherms (8).

The present study corroborates previous work which has shown that muscarinic receptor binding is significantly decreased in the neostriatum of HD brains (4-6). Because we used [^{3}H]QNB of a higher specific activity than that used in previous studies, we have been able to detect binding in areas with relatively low receptor densities (i.e., in several cerebellar regions).

Choline acetyltransferase activity is highest in the corpus striatum with lower and relatively uniform levels in all other regions examined. There is a significant reduction of ChAc activity in the corpus striatum of the HD brains verifying the results of previous studies (6, 28-31). The decreases in [^{3}H]QNB binding and in ChAc activity in HD brains appear not to be due to nonspecific post-mortem changes (32).

Because the $K_{D\ app}$ varies with the receptor concentration (due to the increasing removal of free ligand from the assay medium with increasing amounts of receptor—see ANALYSIS OF BINDING DATA), we did saturation studies at several different receptor concentrations in the putamen, the frontal cortex and the hippocampus. The true K_D values for the control regions were between 20 and 40 pM. These values were in good agreement with those obtained from kinetic measurements. The true K_D for the choreic putamen was not significantly different from the true K_D values determined for the control brain regions. These data, plus that from the dexetimide inhibition studies, indicate that the decrease in muscarinic receptor binding in HD brains is not due to a change in receptor affinity but rather to a decrease in the number of receptors.

Studies, such as these, of other receptors should lead not only to a better understanding of the theoretical significance of receptor binding assays but also to the development of therapies for diseases, like HD, that involve specific receptor deficits.

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