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Quantitative autoradiography of brain binding sites for the vesicular acetylcholine transport blocker 2-(4-phenylpiperidino)cyclohexanol (AH5183)

(digital subtraction autoradiography/*in vitro* binding/cholinergic terminals/basal ganglia/cortex)

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ABSTRACT **2-(4-Phenylpiperidino)cyclohexanol (AH5183) is a noncompetitive and potent inhibitor of high-affinity acetylcholine transport into cholinergic vesicles. It is reported here that [^{3}H]AH5183 binds specifically and saturably to slide-mounted sections of the rat forebrain (K_d = 1.1 to 2.2 × 10^{-8} M; B_{max} = 286 to 399 fmol/mg of protein). The association and dissociation rate constants for [^{3}H]AH5183 binding are 8.6 × 10^6 $M^{-1}\cdot min^{-1}$ and 0.18 min^{-1}, respectively. Bound [^{3}H]AH5183 can be displaced by nonradioactive AH5183 and by the structural analog (2α,3β,4Aβ,8Aα)-decahydro-3-(4-phenyl-1-piperidinyl)-2-naphthalenol but not by 10 μM concentrations of the cholinergic drugs acetylcholine, choline, atropine, hexamethonium, eserine, or hemicholinium-3 or by the structurally related compounds 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 1-methyl-4-phenylpyridine, (±)-*N*-allylnormetazocine (SKF 10,047), levoxadrol, or dexoxadrol. Quantitative autoradiography reveals that [^{3}H]AH5183 binding sites are distributed heterogeneously throughout the rat forebrain and are highly localized to cholinergic nerve terminal regions. At the level of the caudate nucleus–putamen, the highest concentrations of saturable [^{3}H]AH5183 binding (713–751 fmol/mg of protein) are found in the vertical limb of the diagonal band and the olfactory tubercle, with lesser amounts (334–516 fmol/mg of protein) in the caudate–putamen, nucleus accumbens, superficial layers of the cerebral cortex, and the primary olfactory cortex. At day 7 after transsection of the left fimbria, [^{3}H]AH5183 binding and choline acetyltransferase activity in the left hippocampus were reduced by 33 ± 6% and 61 ± 7%, respectively. These findings indicate that [^{3}H]AH5183 binds to a unique recognition site in rat brain that is topographically associated with cholinergic nerve terminals.**

Brittain and coworkers (1, 2) reported that 2-(4-phenylpiperidino)cyclohexanol (AH5183), a structural isomer of hydroxylated phencyclidine, inhibits the coaxially stimulated contractions of the isolated guinea pig ileum. Because AH5183 did not affect the contractions elicited by the addition of acetylcholine (AcCho) to the bath, it was proposed that AH5183 produces a "selective prejunctional inhibition" of cholinergic neurotransmission (1, 2). Based on the pharmacological characteristics of the neuromuscular blockade produced by AH5183 *in vivo* (1, 2), *in vitro*, and *in situ* (3–5), Marshall hypothesized that AH5183 inhibits the loading of AcCho into synaptic vesicles. In support of Marshall's hypothesis, synaptic vesicles isolated from the electric organ of *Torpedo californica*, a model system for the mammalian cholinergic synapse (6), have been shown to acquire AcCho by an active transport process that is pharmacologically distinct from other cholinergic systems (7). AH5183 is the most potent inhibitor of this transport system, half inhibiting AcCho uptake at 40 nM in a noncompetitive manner (7, 8). AH5183 also has been found to inhibit the uptake of newly synthesized AcCho into synaptic storage vesicles in PC12 cells (9, 10) and, presumably as a secondary effect, to inhibit the release of AcCho from the cat superior cervical ganglion *in situ* (11) and from rat and mouse brain preparations *in vitro* (12–14). Remarkably, the above studies could not demonstrate significant direct effects of AH5183 on high-affinity choline uptake, choline acetyltransferase (ChoAcTase) activity, Ca^{2+} influx, or the vesicular content of previously loaded AcCho.

Tritium-labeled AH5183 binds to a site in *Torpedo* vesicles that is saturable, of high affinity (K_d = 3.4 × 10^{-8} M), selective for the *l*-enantiomer of the drug, and not occupied by AcCho or choline (15). The existence of a vesicular binding site for [^{3}H]AH5183 in mammalian peripheral or central cholinergic nerve terminals may explain the ability of AH5183 to prevent the accumulation of AcCho in these preparations. The present experiments demonstrate that a specific binding site for [^{3}H]AH5183 also exists in rat brain. We have examined the pharmacology and the kinetic and equilibrium properties of [^{3}H]AH5183 binding to slide-mounted coronal sections of the rat forebrain. Using quantitative autoradiography, the selectivity of [^{3}H]AH5183 for cholinergic nerve terminals was tested by examining the regional distribution of binding sites in brain sections and by assessing the loss of binding sites in the hippocampus after transsection of the septohippocampal cholinergic pathway.

MATERIALS AND METHODS

Materials. The following chemicals were used in this study: AcCho chloride (Fluka); atropine sulfate, choline chloride, eserine sulfate, hemicholinium-3, and hexamethonium bromide (Sigma); (±)-*N*-allylnormetazocine (SKF 10,047) (Smith Kline & Beckman); and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine ($MePhH_4Py$) and 1-methyl-4-phenylpyridine ($MePhPy^+$) (Research Biochemicals, Wayland, MA). [^{3}H]AH5183 (15) and (2α,3β,4Aβ,8Aα)-decahydro-3-(4-phenyl-1-piperidinyl)-2-naphthalenol (DPPN) were synthesized by S.M.P. Levoxadrol and dexoxadrol were synthesized by R. Dziemian (CIBA–Geigy, Summit, NJ). The AH5183 and DPPN were first solubilized in a small volume

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Abbreviations: Cho, choline; AcCho, acetylcholine; AH5183, 2-(4-phenylpiperidino)cyclohexanol; ChoAcTase, choline acetyltransferase; DPPN, (2α,3β,4Aβ,8Aα)-decahydro-3-(4-phenyl-1-piperidinyl)-2-naphthalenol; SKF 10,047, (±)-*N*-allylnormetazocine; $MePhPy^+$, 1-methyl-4-phenylpyridine; $MePhH_4Py$, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

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of absolute ethanol and then in 50 mM Tris buffer (pH 7.6 at 60°C. All other drugs were dissolved in the Tris buffer.

[^{3}H]AH5183 Binding Assay. Male Sprague–Dawley rats (180–200 g, Marland Farms, Hewitt, NJ) were decapitated, and the brains were removed and frozen rapidly in isopentane at −20°C. For each brain, 24–36 consecutive 20-μm-thick coronal sections located 8.0–8.5 mm anterior to the interaural plane (16) were cut, slide-mounted, stored, and dried as described (17). The sections were prewashed for 10 min at 23°C in a 50 mM Tris buffer (pH 7.6) that contained 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. The sections were transferred to Coplin jars containing 40 ml of the buffer and 0.3–121 nM [^{3}H]AH5183 (32.7 Ci/mmol, 1 Ci = 37 GBq) and were incubated at 23°C for 60 min. The binding of [^{3}H]AH5183 in the presence of other drugs was determined in neighboring sections. Nonspecific binding of [^{3}H]AH5183 was determined in alternate sections with 10 μM *dl*-AH5183. After the incubation, all sections received two rinses of 30-sec duration, each in 4°C buffer, and one 2-sec rinse in 4°C distilled water.

The binding of [^{3}H]AH5183 to brain sections was quantified by swabbing or autoradiographic procedures. For swabbing experiments, duplicate sections of each brain were swabbed off the slides with Whatman GF/B filters immediately after the water rinse. The filters were placed in vials containing 6 ml of Aquasol-2 (DuPont–New England Nuclear, Boston, MA) for liquid scintillation counting of tritium. The fmol of AH5183 bound per mg of protein in the swabbed sections was calculated, based on an average estimate of 0.42 mg of protein (18) contained in 10 randomly selected coronal forebrain sections.

Autoradiography of [^{3}H]AH5183 Binding. After the rinse procedures, the unswabbed sections were dried within 2–4 min by a stream of room-temperature air. The slides were apposed to tritium-sensitive film (LKB [^{3}H]Ultrofilm, Broma, Sweden) in an autoradiography cassette for 3–5 wk at room temperature before developing in Kodak D-19. Tritium standards (19) prepared with [^{3}H]thymidine were included with each sheet of film. The autoradiographs were analyzed by computer-assisted video image analysis and densitometry as described (17).

K_d and B_{max} Calculations. Values for B_{max} and K_d were calculated from swabbing and autoradiographic binding data according to the best fit to a parabola by iterative, nonlinear regression analysis (20).

Transsection of the Septohippocampal Pathway. To produce a unilateral destruction of the septohippocampal cholinergic terminals, the left fimbria was transsected (21). The intact right hemisphere served as the control. Seven days after the surgery, the rats were decapitated and the brains were removed. Coronal sections of brain were cut at the level of the hippocampus, ≈3.2 mm anterior to the interaural plane (16). The sections were processed through the [^{3}H]AH5183 binding assay as described above, and regional binding (see Fig. 3 *Lower*) was measured by quantitative autoradiography. Samples of the left and right hippocampus were dissected from the remaining brain tissue and were assayed for ChoAcTase activity (22).

RESULTS

Initial Determination of K_d and Optimization of Binding Conditions. Using nine [^{3}H]AH5183 concentrations (0.3–121 nM) with the buffer conditions described above, we determined that the K_d value for [^{3}H]AH5183 binding to swabbed rat forebrain coronal sections was 33 ± 14 nM (mean ± SEM, n = four brains). The average specific binding of [^{3}H]AH5183 was 58%. Concentrations of [^{3}H]AH5183 that approximated the apparent K_d were used in subsequent experiments.

The amount or percentage of specific [^{3}H]AH5183 binding was not affected by prewashing the sections for 0, 0.5, or 1 hr before incubation (data not shown). Subsequent experiments were performed with a 30-min prewash time. After incubation in [^{3}H]AH5183, rinsing the sections in 4°C Tris buffer for 2 min or longer increased the specific binding of [^{3}H]AH5183 to 90–92% (not shown). Longer rinses (up to 30 min) did not improve binding specificity beyond 92% but did decrease the amount of specific binding. A rinse time of 1 min was chosen for subsequent experiments to retain both a high amount of specific binding and a relatively high specificity of binding (79%). The percentage of total [^{3}H]AH5183 binding displaced by 10 μM unlabeled AH5183 was maximal (79%) at pH 7.8–8.5 (not shown). To maximize specific binding and maintain the integrity of tissue sections, a pH of 7.7 was used in subsequent experiments.

The incubation of sections at 4°C instead of 23°C did not affect the specificity of [^{3}H]AH5183 binding (76–78%) but reduced the amount of specific binding from 179 to 100 fmol/mg of protein (Table 1). The omission of added Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- ions from the buffer increased the amount of specific binding by 40–57% but also reduced by 27–30% the binding specificity. For subsequent experiments, incubations were performed at room temperature (23°C) in the presence of added ions.

Kinetic and Equilibrium Properties of [^{3}H]AH5183 Binding. Having established the conditions for maximizing the amount and specificity of [^{3}H]AH5183 binding to rat forebrain sections, we determined the ligand affinity (K_d) and tissue concentration (B_{max}) of these sites from kinetic binding data (dissociation and association experiments) and from equilibrium saturation binding data (Scatchard analyses). In association experiments (Fig. 1 *Upper*), the specific binding of [^{3}H]AH5183 increased rapidly during the first 4 min of incubation and reached equilibrium by 30 min. The association rate constant (k_{+1}) was 8.6×10^6 $M^{-1} \cdot min^{-1}$ when calculated from a pseudo-first-order plot of the time course (Fig. 1 *Upper Inset*). Specific binding to brain sections also showed a rapid dissociation rate (k_{-1}) of 1.8×10^{-1} min^{-1} (Fig. 1 *Lower*). The pseudo-first-order and first-order plots of the time course of association or dissociation of [^{3}H]AH5183 were linear, which suggests that this is a bimolecular reaction obeying simple mass-action kinetics. The equilibrium dissociation constant, or K_d, calculated from the kinetic rate constants (k_{-1}/k_{+1}) was 2.1×10^{-8} M.

Saturation analysis of the specific binding of [^{3}H]AH5183 determined under optimal conditions (Fig. 2) revealed a K_d of $1.1 \pm 0.1 \times 10^{-8}$ M and a B_{max} of 286 ± 19 fmol/mg of protein when the untransformed data were analyzed by an iterative curve-fitting procedure (20). These K_d and B_{max} values were similar to those derived by Scatchard analysis of the data ($2.2 \pm 0.4 \times 10^{-8}$ M and 399 ± 41 fmol/mg of protein, respectively) (Fig. 2 *Inset*).

Pharmacological Properties of [^{3}H]AH5183 Binding. The coincubation of rat forebrain sections with 30 nM [^{3}H]AH-

Table 1. Effect of temperature and ions on [^{3}H]AH5183 binding

Incubation conditions		[^{3}H]AH5183 binding, fmol/mg of protein			
°C	Ions*	Total	Non-specific	Specific	% specificity
23	+	238 ± 14	57 ± 7	179 ± 12	76 ± 3
	−	486 ± 33	231 ± 26	252 ± 14	53 ± 3
4	+	126 ± 5	29 ± 2	100 ± 7	78 ± 2
	−	274 ± 10	119 ± 5	157 ± 12	57 ± 3

Values are means ± SEM (n = four brains). Nonspecific binding is defined as [^{3}H]AH5183 binding in the presence of 10 μM unlabeled AH5183.

*Ions are as follows: NaCl (120 mM), KCl (5 mM), $CaCl_2$ (2 mM), and $MgCl_2$ (1 mM).

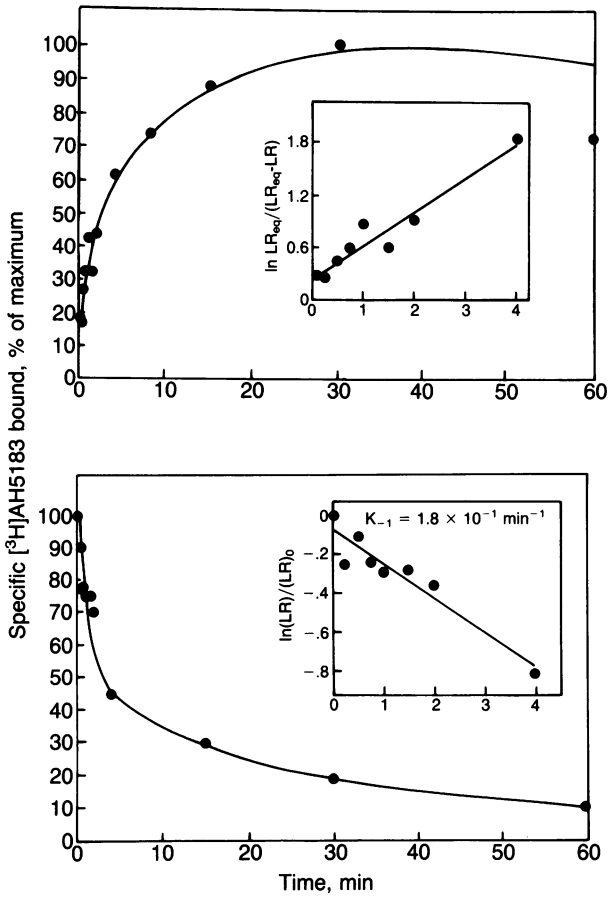

FIG. 1. Kinetic properties of [^{3}H]AH5183 binding to rat forebrain sections. Data are mean values of four brains. The equations are described by Weiland and Molinoff (23). (*Upper*) Time course for the association of [^{3}H]AH5183. Sections were incubated at 23°C with 29.6 nM [^{3}H]AH5183, approximating the initially determined K_d value (3.3×10^{-8} M), for time intervals that ranged from 5 sec to 60 min. (*Inset*) Pseudo-first-order plot of association. LR_{eq} is the concentration of ligand–receptor (LR) complex at equilibrium and LR is the concentration at time t. The slope of the line is equal to k_{+1} (8.6×10^6 M^{-1}·min^{-1}) × L_tR_t/LR_{eq} where $[L]_t$ is the concentration of free ligand and R_t is the B_{max}. (*Lower*) Time course for dissociation. After equilibrium was reached (1-hr incubation at 23°C with 28.7 nM [^{3}H]AH5183), dissociation was initiated by immersing the slide-mounted tissue sections in 200 ml of room-temperature buffer. Rinse intervals ranged from 5 sec to 60 min. (*Inset*) First-order plot of dissociation, where LR is the concentration of ligand–receptor complex at time t, and LR_0 is the concentration at time 0. The slope of the line is equal to $-k_{-1}$ (1.8×10^{-1} min^{-1}). The K_d calculated from k_{-1}/k_{+1} was equal to 2.1×10^{-8} M.

5183 and various cholinergic drugs or compounds structurally related to AH5183 revealed that, with the exception of *dl*-AH5183 and DPPN, specific or total amounts of [^{3}H]AH-5183 binding were not significantly altered by any compound tested. These included 10 μM concentrations of AcCho, choline, atropine, hexamethonium, eserine, hemicholinium-3, SKF 10,047, $MePhH_4Py$, $MePhPy^+$, levoxadrol, and dexoxadrol (data not shown).

Densitometric Analysis of [^{3}H]AH5183 Binding in Autoradiographs. Autoradiographs of rat forebrain sections incubated with [^{3}H]AH5183 revealed a heterogeneous distribution of specific binding sites (Fig. 3). At subsaturating concentrations of 10–40 nM [^{3}H]AH5183, the specificity of regional binding, defined with 10 μM DPPN, averaged between 53% and 83% of the total binding. The highest amount of saturable, DPPN-displaced [^{3}H]AH5183 binding was evident in the vertical limb of the diagonal band, the olfactory tubercle, caudate nucleus–putamen, nucleus ac-

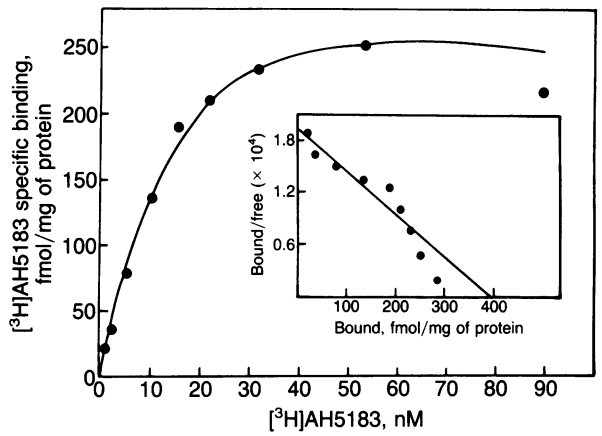

FIG. 2. Saturation analysis of [^{3}H]AH5183 binding (displaced by 10 μM unlabeled *dl*-AH5183) to swabbed sections of rat forebrain. Sections were incubated at room temperature for 60 min with concentrations of [^{3}H]AH5183 ranging from 1.2 to 90 nM and were rinsed for 1 min. Each point represents the mean of four brains. Untransformed data were analyzed by using an iterative curve-fitting computer procedure (20); $K_d = 1.1 \times 10^{-8}$ M and $B_{max} = 286$ fmol/mg of protein. (*Inset*) Scatchard plot of the same data; $K_d = 2.2 \times 10^{-8}$ M and $B_{max} = 399$ fmol/mg of protein.

cumbens, the superficial portion of the cerebral cortex, and the primary olfactory cortex. An increasing medial-to-lateral gradient of binding was evident in the caudate–putamen, while a decreasing medial-to-lateral gradient was apparent in the nucleus accumbens. The K_d values calculated for the eight regions ranged from 12 to 36 nM, and these values were not significantly different between regions (Table 2). The B_{max} values for [^{3}H]AH5183 binding in the diagonal band and olfactory tubercle (751 and 713 fmol/mg of protein, respectively) exceeded the values calculated for the other six regions (334–516 fmol/mg of protein).

[^{3}H]AH5183 Binding in the Hippocampus After Fimbria Transsection. At day 7 after transsection of the left fimbria, [^{3}H]AH5183 binding and ChoAcTase activity in the left hippocampus were reduced by 33 ± 6% and 61 ± 7%, respectively, compared to the right (intact) hippocampus (Table 3). The level of [^{3}H]AH5183 binding in the overlying parietal cortex was the same in both the left and right hemisphere, indicating that the hemispheric differences in hippocampal binding site concentrations were not simply a result of an asymmetric binding or image analysis artifact.

DISCUSSION

The present study demonstrates that [^{3}H]AH5183 binds with high affinity to a saturable and pharmacologically unique site in the rat forebrain. Under the optimized *in vitro* binding assay conditions determined in the preliminary experiments, the K_d value calculated by kinetic or equilibrium binding procedures ranged from 11 to 36 nM, and the dissociation rate for [^{3}H]AH5183 was 1.8×10^{-1} min^{-1}. The kinetic and equilibrium properties of [^{3}H]AH5183 binding to brain sections were virtually identical to those measured in purified cholinergic vesicles of *Torpedo* electric organ ($K_d = 3.4 \times 10^{-8}$ M, dissociation rate constant = 2.3×10^{-1} min^{-1}) (15). These K_d values approximated the IC_{50} value (40 nM) for AH5183 inhibition of vesicular AcCho transport (7).

These remarkably similar kinetic binding values suggest that the [^{3}H]AH5183 binding site in the rat brain may be analogous to the binding site on *Torpedo* cholinergic vesicles. In support of this, the pharmacological specificity of [^{3}H]AH5183 binding to brain sections and *Torpedo* vesicles (15) was similar. [^{3}H]AH5183 binding was displaced by unlabeled AH5183 and by its analog DPPN but was not

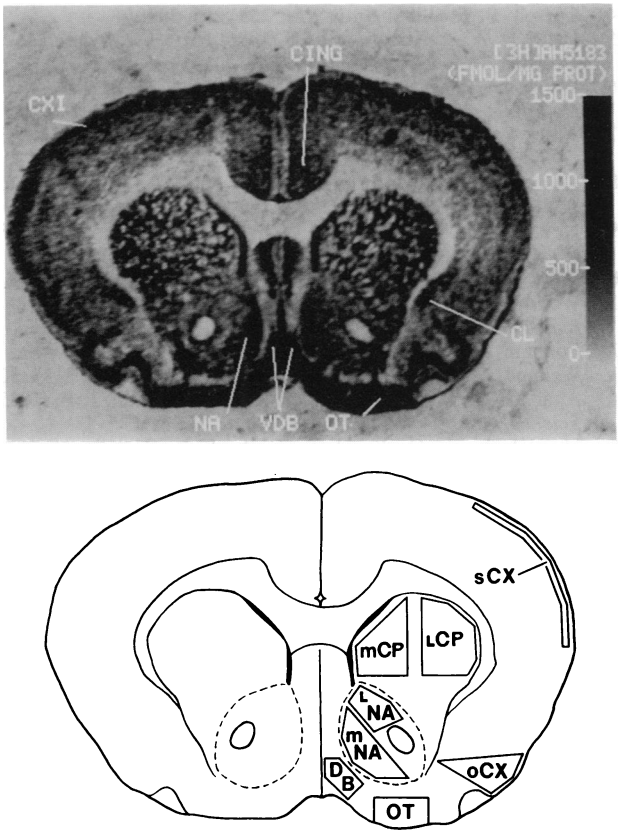

FIG. 3. (*Upper*) Digital subtraction autoradiography (17, 24) reveals specific [^{3}H]AH5183 binding sites in a coronal section of the rat forebrain. The concentrations of [^{3}H]AH5183 present in the tissue are indicated by the gray-value concentration scale to the right of the figure. The image of specific binding was produced by subtracting the "linearized" (17) gray values of the image of nonspecific binding from the superimposed image of total [^{3}H]AH5183 binding to an adjacent brain section. (*Lower*) Typical cursor placements over brain regions in which [^{3}H]AH5183 concentrations were measured for Table 2. DB, vertical limb of the diagonal band; LCP, lateral caudate–putamen; mCP, medial caudate–putamen; LNA, lateral nucleus accumbens; mNA, medial nucleus accumbens; oCX, primary olfactory cortex; OT, olfactory tubercle; sCX, superficial cortex.

affected by 10 μM concentrations of AcCho or choline. In addition, atropine, hexamethonium, eserine, and hemicholinium-3 did not inhibit [^{3}H]AH5183 binding to brain sections. Each of these compounds is also less potent as an inhibitor of AcCho transport by at least a factor of 250 than is AH5183 (7). Thus, it seems unlikely that the [^{3}H]AH5183 binding site in brain corresponds to either a muscarinic or nicotinic receptor, the enzymes ChoAcTase or AcCho esterase, or the high-affinity choline transport system.

Table 2. Regional distribution of [^{3}H]AH5183 binding

Region	K_d × 10^{-8} M	B_{max}, fmol/mg of protein
Caudate–putamen		
Medial	3.6 ± 1.2	410 ± 57
Lateral	3.1 ± 1.1	516 ± 84
Nucleus accumbens		
Medial	2.0 ± 0.1	461 ± 20
Lateral	1.2 ± 0.3	422 ± 29
Olfactory tubercle	1.6 ± 0.3	713 ± 43*
Diagonal band	1.9 ± 0.5	751 ± 91*
Superficial cortex	2.4 ± 0.9	396 ± 39
Olfactory cortex	1.3 ± 0.2	334 ± 39

Values are means ± SEM (n = 4 brains). Specific binding in subcortical regions was 68–83% of total binding; specific binding in cortical regions was 53–62% of the total.
*$P < 0.05$ compared to other brain regions by Dunnett's multiple t test.

Table 3. Reduction of [^{3}H]AH5183 binding sites after cholinergic lesion

Region	AH5183 binding, fmol/mg of protein: Left hemisphere	Right hemisphere	% change
Hippocampus	315 ± 25	477 ± 28	−33 ± 6*
Parietal cortex	499 ± 36	526 ± 24	−5 ± 7

Binding of [^{3}H]AH5183 in the left hippocampus 7 days after transsection of the ipsilateral fimbria was studied. Hippocampal ChoAcTase activity was reduced by 61 ± 7% in the right hemisphere.
*$P < 0.01$, n = five rats.

The pharmacological specificity of the [^{3}H]AH5183 binding site in brain is demonstrated also by the lack of displacement by structurally related drugs. The binding of [^{3}H]AH5183, a structural isomer of hydroxylated phencyclidine, was not affected by dexoxadrol or levoxadrol, which are enantiomers that exhibit stereoselectivity (IC_{50} = 21 and 6100 nM, respectively) for a high-affinity phencyclidine receptor in brain (25). Furthermore, the possibility that [^{3}H]AH5183 recognizes phencyclidine receptors (25) or μ or σ opioid receptors (26) is excluded by our finding that 10 μM (±)SKF 10,047 did not affect [^{3}H]AH5183 binding. The phenylpyridine derivatives $MePhH_4Py$ and $MePhPy^+$ were also ineffective as displacers of [^{3}H]AH5183, even at 10 μM concentrations, which far exceed the low nanomolar K_d values of the specific binding of $MePhH_4Py$ (27, 28) or $MePhPy^+$ (29) in brain. These findings demonstrate a remarkably strict structural specificity of the [^{3}H]AH5183 binding site in brain.

The visualization and measurement of specific [^{3}H]AH-5183 binding sites by autoradiography revealed a heterogeneous distribution throughout the rat forebrain that corresponded closely to the distribution of markers for cholinergic neurons. For example, within the somatosensory cortex, the higher concentrations of [^{3}H]AH5183 binding sites apparent in the more superficial layers (Fig. 3) resembled the cortical distribution of ChoAcTase activity (30) and immunoreactivity (31). Similarly, the distinct medial-to-lateral increase of [^{3}H]AH5183 binding sites observed in the autoradiographic images of the caudate–putamen corresponded with similar gradients of ChoAcTase activity (32) and high-affinity choline uptake (33) measured in microdissected areas of the caudate–putamen. We recently determined (34) that concentrations of [^{3}H]AH5183 binding in 14–18 brain areas correlated highly (r = 0.88 − 0.97) and significantly ($P < 0.01$) with regional levels of ChoAcTase (35, 36), AcCho esterase (16, 37), and AcCho itself (35, 36). The [^{3}H]AH5183 binding site in the forebrain was also strikingly similar to the autoradiographic distribution (38, 39) and pH optimum and binding affinity (38, 40) of [^{3}H]hemicholinium-3, a drug that labels with nanomolar affinity a site associated with the high-affinity choline-uptake system. That 10 μM hemicholinium-3 did not displace [^{3}H]AH5183 demonstrates that [^{3}H]AH5183 and [^{3}H]hemicholinium-3 label pharmacologically distinct sites.

The conclusion that the [^{3}H]AH5183 binding site is closely associated with cholinergic nerve terminals is supported by the finding that partial destruction of the septohippocampal cholinergic terminal field, assessed by a 61% loss of hippocampal ChoAcTase activity, was accompanied by a 33% loss of [^{3}H]AH5183 binding sites in the hippocampus. The reason for the unequal losses in ChoAcTase activity and [^{3}H]AH5183 binding sites 7 days after the lesion is unclear.

Interestingly, however, destruction of cholinergic afferents to the rat cortex result in a similar discrepancy between cortical ChoAcTase loss and [^{3}H]hemicholinium-3 binding as measured by quantitative autoradiography (39). The discrepancy in the present study may be attributable to the persistence of the binding sites within degenerating terminals, the presence of [^{3}H]AH5183 binding sites on noncholinergic structures, or differences in the disappearance rates of ChoAcTase and [^{3}H]AH5183 binding sites.

Thus, it appears that a specific binding site for AH5183 is present in rat brain, and the occupation of this site by AH5183 could account for the block of AcCho release from *in vitro* brain preparations observed by others. This putative receptor may represent a unique intracellular mechanism by which cholinergic activity can be modulated at the level of AcCho transport into vesicles. The use of [^{3}H]AH5183 autoradiography to localize and quantify this site represents a direct approach for the investigation of this potentially important component of cholinergic neuronal function.

1. Brittain, R. T., Levy, G. P. & Tyers, M. B. (1969) *Br. J. Pharmacol.* **36,** 173–174.
2. Brittain, R. T., Levy, G. P. & Tyers, M. B. (1969) *Eur. J. Pharmacol.* **8,** 93–99.
3. Marshall, I. G. (1970) *Br. J. Pharmacol.* **38,** 503–516.
4. Marshall, I. G. (1970) *Br. J. Pharmacol.* **40,** 68–77.
5. Gandiha, A. & Marshall, I. G. (1973) *Int. J. Neurosci.* **5,** 191–196.
6. Whittaker, V. P. & Zimmermann, H. (1976) in *Biochemical and Biophysical Perspectives in Marine Biology*, eds. Malins, D. C. & Sargent, J. R. (Academic, London), pp. 67–116.
7. Anderson, D. C., King, S. C. & Parsons, S. M. (1983) *Mol. Pharmacol.* **24,** 48–54.
8. Bahr, B. A. & Parsons, S. M. (1986) *J. Neurochem.* **46,** 1214–1218.
9. Toll, L. & Howard, B. D. (1980) *J. Biol. Chem.* **255,** 1787–1789.
10. Melega, W. P. & Howard, B. D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6536–6538.
11. Collier, B., Welner, S. A., Ricny, J. & Araujo, D. M. (1986) *J. Neurochem.* **46,** 822–830.
12. Otero, D. H., Wilbekin, F. & Meyer, E. M. (1985) *Brain Res.* **359,** 208–214.
13. Jope, R. S. & Johnson, G. V. W. (1985) *Mol. Pharmacol.* **29,** 45–51.
14. Carroll, P. T. (1985) *Brain Res.* **358,** 200–209.
15. Bahr, B. A. & Parsons, S. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2267–2270.
16. Paxinos, G. & Watson, C. (1982) *The Rat Brain in Stereotaxic Coordinates* (Academic, New York).
17. Altar, C. A., Walter, R. J., Neve, K. A. & Marshall, J. F. (1984) *J. Neurosci. Methods* **10,** 173–188.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
19. Neve, K. A., Altar, C. A., Wong, C. A. & Marshall, J. F. (1984) *Brain Res.* **302,** 9–18.
20. Bliss, C. I. & James, A. T. (1966) *Biometrics* **22,** 573–602.
21. Wood, P. L., Cheney, D. L. & Costa, E. (1979) *J. Pharmacol. Exp. Ther.* **209,** 97–103.
22. Fonnum, F. (1975) *J. Neurochem.* **24,** 407–409.
23. Weiland, G. A. & Molinoff, P. B. (1981) *Life Sci.* **29,** 313–330.
24. Altar, C. A., O'Neil, S., Walter, R. J. & Marshall, J. F. (1985) *Science* **228,** 597–600.
25. Mendelsohn, L. G., Kerchner, G. A., Kalra, V., Zimmerman, D. M. & Leander, J. D. (1984) *Biochem. Pharmacol.* **33,** 3529–3535.
26. Su, T.-P. (1982) *J. Pharmacol. Exp. Ther.* **223,** 284–290.
27. Javitch, J. A., Uhl, G. A. & Snyder, S. H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4591–4595.
28. Wieczorek, C. M., Parsons, B. & Rainbow, T. C. (1984) *Eur. J. Pharmacol.* **98,** 453–454.
29. Altar, C. A., Heikkila, R. E., Manzino, L. & Marien, M. R. (1986) *Eur. J. Pharmacol.*, in press.
30. Beesley, P. W. & Emson, P. C. (1975) *Biochem. Soc. Trans.* **3,** 936–939.
31. Houser, C. R., Crawford, G. D., Salvaterra, P. M. & Vaughn, J. E. (1985) *J. Comp. Neurol.* **234,** 17–34.
32. Guyenet, P., Euvrard, C., Javoy, F., Herbet, A. & Glowinski, J. (1977) *Brain Res.* **136,** 487–500.
33. Rea, M. A. & Simon, J. R. (1981) *Brain Res.* **219,** 317–326.
34. Marien, M. R., Parsons, S. M. & Altar, C. A. (1986) *Soc. Neurosci. Abstr.* **12,** 239.
35. Cheney, D. L., LeFevre, H. F. & Racagni, G. (1975) *Neuropharmacology* **14,** 801–809.
36. Hoover, D. B., Muth, E. A. & Jacobowitz, D. M. (1978) *Brain Res.* **153,** 295–306.
37. Satoh, K., Armstrong, D. M. & Fibiger, H. C. (1983) *Brain Res. Bull.* **11,** 693–720.
38. Rainbow, T. C., Parsons, B. & Wieczorek, C. M. (1984) *Eur. J. Pharmacol.* **102,** 195–196.
39. Pilapil, C. & Quirion, R. (1985) *Soc. Neurosci. Abstr.* **11,** 665.
40. Sandberg, K. & Coyle, J. T. (1985) *Brain Res.* **348,** 321–330.