Neurochemical Research, Vol. 14, No. 8, 1989, pp. 745–750

Nicotinic Binding Sites in Cerebral Cortex and Hippocampus in Alzheimer's Dementia

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(Accepted May 8, 1989)

Postmortem cerebral neocortical and hippocampal samples were taken from patients who died with dementia of the Alzheimer type (DAT) and individuals without diagnoses of neurological or psychiatric disease (control). Nicotinic binding was assayed with 20 nM [^{3}H]acetylcholine ([^{3}H]ACh) in the presence of atropine, or with 4 nM (-)-[^{3}H]nicotine ((-)-[^{3}H]Nic). Binding of both ligands was lower in the following regions from DAT vs. control brains ($P \leq 0.05$): superior, middle and inferior temproal gyri, orbital frontal gyrus, middle frontal gyrus, pre- and postcentral gyri, inferior parietal lobule, and hippocampal endplate. Values of the correlation coefficient (r's) for binding of the nicotinic cholinergic ligands in these regions ranged from 0.70 to 0.93 (P's < 0.05), suggesting that [^{3}H]ACh and (-)-[^{3}H]Nic labeled the same sites in human brain. There was no difference in nicotinic binding in the presubiculum, comparing DAT and control samples ($P > 0.05$). Here too, correlations between binding of the two ligands were statistically significant in control and DAT groups (r's $= 0.92$, P's < 0.05). Nicotinic binding measured with [^{3}H]ACh, but not (-)-[^{3}H]Nic, was significantly lower in the H_2 (field of Rose) and H_1-subiculum areas of DAT samples compared to control. Correlations between binding of the two ligands in these regions ranged from 0.21 to 0.34 for the two groups (P's > 0.05). The findings support a loss of neocortical and hippocampal nicotininc cholinergic binding sites in DAT. Further study is necessary to better characterize the regional losses of nicotinic binding in DAT and to resolve the differences in binding measured by [^{3}H]ACh and (-)-[^{3}H]Nic in the H_1-subiculum and H_2 (field of Rose) regions.

KEY WORDS: Dementia of the AlzheimerType; neocortex nicotinic acetylcholine receptors; hippocampus.

INTRODUCTION

Dementia of the Alzheimer type (DAT) is characterized clinically by a progressive deterioration of cognitive and mental functions (1). Many neuronal systems have been implicated in DAT (2, 3), and substantial evidence suggests a cholinergic abnormality (3-7). The most prominent neurochemical finding is a marked reduction of acetyl-CoA: choline-O-acetyltransferase (EC 2.3.1.6, choline acetyltransferase) activity (2, 4, 5, 8, 9), which has been correlated with the degree of cognitive impairment and histopathological change in DAT (9-12). However, despite consistent decrements of choline acetyltransferase activity in DAT, studies of muscarinic binding have provided conflicting results. Some studies demonstrated no significant differences compared with control (11, 13-19), although significant reductions (6, 20-28) and increases (19, 26, 27) in the

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0364-3190/89/0800-0745$06.00/0

density of muscarinic binding sites have also been noted in association with DAT.

Reductions in nicotinic cholinergic binding sites in DAT have been reported using [^{3}H]acetylcholine ([^{3}H]ACh), (-)-[^{3}H]nicotine ((-)-[^{3}H]Nic), or [^{3}H]methylcarbamylcholine in samples from the putamen, nucleus basalis of Meynert, hippocampus, and frontal, temporal, parietal and occipital poles of the cerebral cortex (20, 28-32). The purpose of the present study was to extend previous findings on nicotinic receptor losses in DAT, using a fine regional dissection of the cerebral cortex and hippocampal formation and a comparison of two radioligands.

EXPERIMENTAL PROCEDURE

Brain tissue was taken at autopsies of 42 individuals (28 men and 14 women) from the participating cohort of the Dementia Study Group of the University of Western Ontario. The cause of death in the majority of patients was broncho-pneumonia and/or myocardial infarction. Tissue used in the necropsy examination and all subsequent biochemical studies was available usually within 10-12 h after death, and frequently within 2-4 h. Patients diagnosed as demented had significant and generally quite severe cognitive impairment prior to death.

Non-neurological controls were judged to be mentally intact on the basis of all available medical records. The clinical diagnosis of DAT was confirmed or modified by necropsy examination of the left hemisphere, as described previously (10). The diagnosis of DAT (n = 26; 14 men and 12 women) was confirmed by the presence of extensive formation of neuritic plaques and neurofibrillary tangles as well as the absence of infarctions, Pick inclusion bodies, Parkinson's disease or other organic causes for a dementing illness. The severity of plaque and tangle formation met the quantitative histopathologic criteria proposed by the National Institutes of Health workshop (33); and the density of hippocampal lesions also met the diagnostic criteria promulgated by the Dementia Study Laboratory, University of Western Ontario (34). All DAT patients were diagnosed as severely demented and in an advanced phase of illness. Sixteen cases (14 men and 2 women), which had no clinical or necropsy evidence of a dementia and no neurological or psychiatric, difficulties served as controls. The mean age ±SD (range) in years for subjects in the DAT group was 75.9 ± 7.9 (60-89) and in the control group was 61.6 ± 12.9 (32-75).

With the exception of 5 control subjects, individuals in this study were undergoing treatment with antipsychotics, antidepressants, or centrally-acting anticholinergics at the time of or prior to their deaths. In addition, five individuals included in the present study (all DAT cases) had a history of alcohol abuse, and 6 individuals (1 DAT and 5 control) had suffered from diabetes mellitus at the time of their deaths. The potential confounding effects of age, diabetes, alcohol abuse and drug treatments on nicotinic binding were assessed separately within each diagnostic group comparing subjects with positive and negative histories. Tests of significance for the paired comparisons were based on at least squares solution. With the small number of subjects with a history of diabetes, alcohol abuse or drug treatments, and their uneven distribution across diagnostic groups, no significant differences could be demonstrated due to age, diabetes, alcohol abuse or drug treatment ($p > 0.05$).

Tissue for biochemical assay was removed from a total of 12 neocrotical and hippocampal-limbic regions of the right hemisphere of each individual. Subsectioning of the hippocampus corresponded to the anatomical dissection described in detail previously (35). Immediately after the dissection, the brain samples were either rapidly deep frozen to −70°C or sonicated in 20 volumes (wt/vol, 1 mg/20 μl) of glass distilled water, then deep frozen to −70°C and kept at this temperature until the time of assay.

Nicotinic cholinergic binding was measured using a modification of the methods of Schwartz and Kellar (36) and Schwartz et al. (37). Sonicates were centrifuged at 35,000 *g* for 15 min. The pellet was resuspended in 50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ (pH 7.44 at 0°C). The incubation buffer for assays using [^{3}H]ACh also contained 1.5 μM atropine, a muscarinic antagonist, and 300 μM diisopropylfluorophosphate, a cholinesterase inhibitor. The mixture was centrifuged at 35,000 *g* for 15 minutes, the supernatant fluid discarded, and the pellet resuspended in the Tris-HCl-salt buffer containing diisopropylfluorophosphate. Aliquots of homogenates (~0.8-1.2 mg original wet weight tissue) were incubated at 0°C for 60 min with 20 nM [^{3}H]ACh (86 Ci/mmol, Amersham Corp., Arlington Heights, IL, U.S.A.) or 4 nM (-)-[^{3}H]Nic (71.9 Ci/mmol, New England Nuclear, Boston, MA, U.S.A.). Figure 1 presents representative Scatchard plots for both ligands obtained in a separate series of studies using control human temporal gyrus. In these studies, an average K_d of 6.72 ± 1.02 nM (mean ± SEM for 5 determinations) for [^{3}H]ACh and 1.75 ± 0.22 nM for (-)-[^{3}H]Nic were obtained. Thus, the single ligand concentrations used in the present study are 3 to 5 times the K_d values for the ligand. Incubations were terminated by reduced pressure filtration using a Brandel Receptor Harvester (MR-12, Gaithersburg, MD, USA.) through GF/C filters pre-wetted with 0.05% polyethyleneimine. Filters were washed three times with 2-ml aliquots of cold buffer and were counted using liquid scintillation spectrometry. Specific binding was defined as the difference between total and nonspecific binding, assayed in the presence of 100 μM carbamylcholine. Nonspecific binding was subtracted from total binding at the same [^{3}H]ligand concentration. Variation in binding among triplicate samples was always less than 15% of the mean, and the percent of [^{3}H]ligand bound was always less than 3% of the free ligand concentration. Specific binding was expressed as fmol [^{3}H]ligand bound per mg protein. Protein concentration in homogenates was measured using the Bio-Rad Protein Assay (Bio-Rad, Richmond, CA, U.S.A.).

All statistical analyses were performed using the SYSTAT statistical package (Systat Inc., Evanston, IL) on an MS-DOS personal computer system. For each region tested, means calculated for the different diagnoses using each ligand were compared using a two-way analysis of variance, with the diagnosis (DAT, control) and radioligands ([^{3}H]ACh and (-)-[^{3}H]Nic) as the main effects. The significance of differences between individual means was assessed by Duncan's New Multiple Range test. Correlation coefficients (r's) were also calculated by linear regression to determine relationships between binding of the two radioligands for each region and diagnosis. The criterion for significance was $p < 0.05$.

RESULTS

The binding of 20 nM [^{3}H]ACh and 4 nM (-)-[^{3}H]Nic to nictoinic cholinergic binding sites in regions from the hippocampus and neocortex is shown in Table 1. Ni-

Table I. The binding of [^{3}H]ACh (20 nM, in the Presence of Atropine and DFP) and (-)-[^{3}H]Nic (4 nM) in Twelve Brain Regions from Right Hemispheres of Control and DAT Patients

	CONTROL (n = 16)		DAT (n = 26)	
	[^{3}H]ACh	[^{3}H]Nic	[^{3}H]ACh	[^{3}H]Nic
Hippocampal Areas				
Endplate	13.05 ± 0.7[a]	15.03 ± 0.8	7.13 ± 0.6*	8.31 ± 0.8*
H_2 Field of Rose	9.41 ± 1.0	13.12 ± 1.1	4.24 ± 1.2*	13.02 ± 2.0†
H_2-Subiculum	12.54 ± 1.0	16.45 ± 1.1	7.34 ± 0.9*	14.32 ± 0.8†
Presubiculum	16.75 ± 0.7	16.75 ± 0.6	15.3 ± 0.4	14.3 ± 0.3
Neocortical Areas				
Frontal Gyri				
Precentral	13.67 ± 1.1	11.67 ± 0.8	9.01 ± 1.0*	7.05 ± 0.7*
Orbital	14.78 ± 1.3	11.56 ± 0.8	7.01 ± 1.0*	5.78 ± 1.1*
Middle	10.98 ± 1.0	9.98 ± 1.0	5.23 ± 0.9*	5.66 ± 0.7*
Temporal Gyri				
Superior	13.67 ± 0.5	13.89 ± 0.6	9.45 ± 0.3*	9.32 ± 0.3*
Middle	15.04 ± 1.3	13.87 ± 2.7	8.98 ± 1.9*	9.54 ± 0.4*
Inferior	9.87 ± 2.1	7.97 ± 0.5	5.61 ± 0.7*	4.55 ± 0.6*
Parietal Cortex				
Postcentral Gyrus	13.67 ± 1.1	11.67 ± 0.8	9.00 ± 0.8*	7.05 ± 0.7*
Inferior Lobule	12.00 ± 0.8	11.52 ± 0.5	7.54 ± 0.4*	7.30 ± 0.4*

[a] Mean ± S.E.M. Specific binding expressed as fmoles [^{3}H]ligand bound per mg protein.
* significantly different from control binding measured with same ligand, $p \leq 0.05$.
† significantly different from DAT binding measured with [^{3}H]ACh, $p \leq 0.05$.

cotinic cholinergic binding in the hippocampal endplate was significantly lower by 46% and 44% in DAT samples compared with control, using [^{3}H]ACh and (-)-[^{3}H]Nic, respectively. In the H_2 (field of Rose) and H_1-subiculum areas from DAT vs. control subjects, nicotinic cholinergic binding measured by [^{3}H]ACh, but not by (-)-[^{3}H]Nic, was significantly lower. There were no statistically significant differences in (-)-[^{3}H]Nic binding observed between DAT and control in these two regions. Further, comparison of binding measured by each ligand for each diagnosis revealed statistical differences between the binding of [^{3}H]ACh and (-)-[^{3}H]Nic in H_2 (field of Rose) and H_1-subiculum samples obtained from DAT individuals but not from control samples. In DAT samples, the binding of (-)-[^{3}H]Nic was 68% and 49% lower, respectively, compared to the binding of [^{3}H]ACh. Nicotinic binding was lower by 9% and 14% in the presubiculum of DAT patients compared to control [^{3}H]ACh and (-)-[^{3}H]Nic, respectively; the differences were not statistically significant.

For each of the neocortical regions tested, ligand binding to nicotinic cholinergic sites was significantly lower in samples from DAT patients than in controls. The reduction in nicotinic binding ranged from 53% in the orbital frontal gyrus ([^{3}H]ACh) to 31% in the middle temporal gyrus ((-)-[^{3}H]Nic). The average loss of nicotinic binding in all regions of DAT brain vs. control samples assayed was 40% using [^{3}H]ACh and (-)-[^{3}H]Nic as the ligands.

The correlations between the binding of [^{3}H]ACh and (-)-[^{3}H]Nic in the twelve brain regions were also tested. With the exception of the H_2 (field of Rose) and H_1-subiculum regions, the correlations between the two nicotinic cholinergic ligands were significantly positive for DAT and control tissues (r's ranged from 0.69 to 0.93). In the H_2 (field of Rose) and H_1-subiculum, correlations were low and statistically nonsignificant (r's ranged from 0.21 to 0.34) for both control and DAT tissues.

DISCUSSION

The present findings confirm and extend earlier reports of altered nicotinic binding in cortical and hippocampal brain regions with DAT (20, 23, 28, 31, 32). New information is provided regarding the regional losses of cholinergic nicotinic binding sites associated with DAT and the correlation between nicotinic binding measured by (-)-[^{3}H]Nic and [^{3}H]ACh. Previous reports on the densities of nicotinic binding sites in postmortem brains from demented patients had shown either no difference

or a lower density as compared with values in non-neurological controls. A reduction in central nicotinic binding sites labelled with [^{125}I]α-bungarotoxin was reported in the middle temporal gyrus of demented patients (38). However, this finding was not confirmed (16). Evidence has been presented that α-bungarotoxin does not block central nicotinic function, and therefore may not be a suitable ligand to assay central nicotinic cholinergic binding (39, 40). Further, several studies have demonstrated regional differences in the binding of radiolabeled α-bungarotoxin compared with radiolabeled acetylcholine and nicotine, which show similar profiles of central nicotinic binding (41–43). The ligands [^{3}H]ACh, (-)-[^{3}H]Nic, and [^{3}H]methylcarbamylcholine have been useful for the characterization of central nicotinic cholinergic binding sites (20, 36, 37, 41, 43, 44). Araujo et al. (20) reported reduced binding of [^{3}H]methylcarbamylcholine in frontal, temporal, parietal, and occipital cortical and hippocampal samples from DAT brains, but no difference in the striatum, globus pallidus, thalamus and nucleus basalis of Meynert. Using [^{3}H]ACh to determine nicotinic binding, several groups have reported reduced binding associated with DAT in the frontal, temporal and occipital cortices (28, 29, 31, 32), although in one report (32), the reduction of nicotinic binding in the occipital cortex was not statistically significant. Significant reductions of [^{3}H]Nic binding in the putamen and nucleus basalis of Meynert, and no difference in the frontal and temporal cortices, hippocampus, caudate nucleus, thalamus, and cerebellum have been noted in demented brains (30). Other studies using [^{3}H]Nic to determine central nicotinic binding in dementia have shown statistically significant reductions in the frontal, temporal and occipital cortices (29, 31).

We observed reduced nicotinic binding, determined using [^{3}H]ACh and (-)-[^{3}H]Nic, in several regions of the neocortex and hippocampus from DAT brains. However, we observed no change in nicotine binding in the presubiculum as determined with both ligands, or in the H_2 (field of Rose) and H_1-subiculum when using (-)-[^{3}H]Nic. Nicotinic binding in the latter two regions determined using [^{3}H]ACh was significantly lower in DAT. Preliminary Scatchard analysis of the binding of [^{3}H]ACh and (-)-[^{3}H]Nic using regional brain samples pooled from several control and DAT subjects suggests that the changes reported here are associated with a change in B_{max} and not a change in K_d (Figure 1).

Our investigation of nicotinic binding in DAT suggests that while there are several regionally specific changes associated with dementia, they are not as widespread or consistent as the well-documented changes in choline acetyltransferase. Using these same samples, a

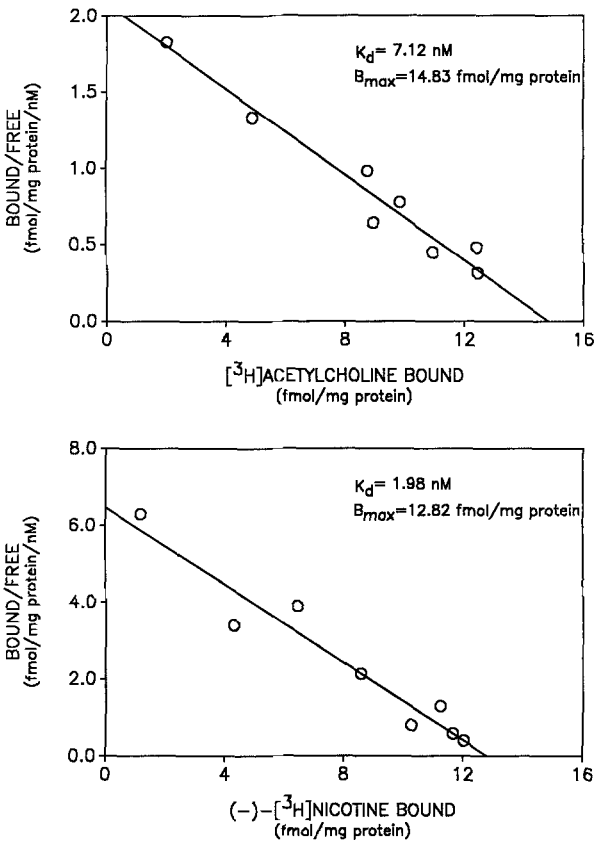

Fig. 1. Scatchard plots of [^{3}H]acetylcholine (top) and (-)-[^{3}H]nicotine (bottom) to nicotinic binding sites in human temporal cortex for 1 control brain. Binding of [^{3}H]ACh (1–40 nM) and (-)-[^{3}H]Nic (0.2–30 nM) to nicotinic binding sites as described in Experimental Procedure with the exception of a varied ligand concentration.

robust and consistent loss of choline acetyltransferase was observed in each of the twelve regions tested here (19; unpublished results (postcentral gyrus and inferior parietal lobule)). However, the loss of nicotine binding sites in DAT was observed in only 11 regions using [^{3}H]ACh and only nine regions using (-)-[^{3}H]Nic. Partly because of the parallel reductions between choline acetyltransferase and nicotinic binding in brains from patients who died with DAT, it has been proposed that the nicotinic binding sites lost in dementia are largely presynaptic (20, 29, 31). The present study did not examine the correlation between choline acetyltransferase activity and nicotinic binding; however the fact that nicotinic binding measured by [^{3}H]ACh or [^{3}H]nicotine was not reduced in all regions while choline acetyltransferase was reduced suggests that nicotinic binding sites may be postsynaptic in the presubiculum and, possibly, the H_2 (field of Rose) and H_1-subiculum areas.

Whitehouse et al. (31) reported statistically significant positive correlations between the binding of [^{3}H]ACh and (-)-[^{3}H]Nic in control tissues, suggesting that these ligands label the same nicotinic recognition sites in human brain tissue. An autoradiographic study in rat brain has also suggested that the two radioligands label the same sites (41). We also observed statistically significant positive correlations for ten of the twelve regions tested in both control and DAT tissues. However, in the H_2 (field of Rose) and the H_1-subiculum, the correlations between [^{3}H]ACh and (-)-[^{3}H]Nic binding were not statistically significant. The lack of correlation suggests that the original proposal by Whitehouse et al. (31) and Araujo et al. (20) may not be true for all regions of the brain, and that the H_2 and H_1-subiculum show greater heterogeneity of nicotinic receptors than the other regions assayed. Heterogeneity of cerebral nicotinic binding has been demonstrated using rats by Larsson et al. (45), who reported that subchronic treatment of rats with nicotine increased [^{3}H]ACh but not [^{3}H]Nic binding in the midbrain and hippocampus.

ACKNOWLEDGMENTS

This work was supported in part by grants to M.J. Ball from the National Institutes of Health (AG03047-06), the Medical Research Council of Canada (PG-21) and the Atkinson Charitable Foundation of Toronto and by grants to S.B. Waller from the American Federation for Aging Research, the University of South Dakota School of Medicine Parson's Research Fund, and the National Institute on Aging (AG06421). The authors gratefully acknowledge the assistance of the Dementia Study Group, University of Western Ontario.

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