Muscarinic Binding and Choline Acetyltransferase in Postmortem Brains of Demented Patients

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ABSTRACT: Postmortem human brain samples were taken from non-neurological controls as well as demented subjects who died with Alzheimer's disease (AD), multi-infarct dementia (MID), or a combination of AD and MID dementia (MIXED). Choline acetyltransferase (ChAT) activity was measured radiometrically using [1-^{14}C]acetyl-coenzyme A as the substrate, muscarinic binding was assayed with [^{3}H]quinuclidinyl benzilate, and the proportion of binding associated with high affinity agonist sites was measured by carbamylcholine displacement of the radioligand.

Relative to control, ChAT activity was significantly reduced ($p \leq 0.01$) in samples taken from the temporal, frontal, and hippocampal areas of demented patients. A small elevation in B_{max} was noted in the hippocampal endplate ($p \leq 0.01$) (AD vs. control) and the H_1-subiculum region ($p \leq 0.05$) (AD vs. all other groups). In addition, the percentage of binding associated with high affinity agonist sites was greater in the frontal cortex of AD and MID samples ($p \leq 0.05$), compared with MIXED and control, and in the temporal cortex of the AD group compared with all other groups ($p \leq 0.05$). The results suggest a regionally specific upregulation of cerebral muscarinic receptors in dementia, especially in AD.

RÉSUMÉ: Liaison muscarinique et choline acétyltransférase dans le cerveau de patients déments, obtenus par autopsie. Nous avons obtenu des échantillons de cerveau humain provenant de l'autopsie de témoins, sans affection neurologique, ainsi que de sujets déments atteints de la maladie d'Alzheimer (MA), de démence due à des infarctus multiples (DIM) ou d'une combinaison de démence MA et DIM (MIXTE). Nous avons mesuré l'activité de la choline acétyltransférase par radiométrie en utilisant le [1-^{14}C] acétyl-coenzyme A comme substrat. Nous avons évalué la liaison muscarinique au moyen du [^{3}H] benzilate de quinuclidynil et la proportion de la liaison associée aux sites agonistes de haute affinité par déplacement du radioligand par la carbamylcholine.

L'activité de la ChAT était diminuée de façon significatif ($p \leq 0.01$) par rapport aux témoins dans les échantillons provenant des régions temporale, frontale et de l'hippocampe des patients déments. Nous avons noté une légère élévation du B_{max} dans la plaque terminale de l'hippocampe ($p \leq o.01$) (MA vs témoins) et dans la région H_1-subiculum ($p \leq 0.05$) (MA vs tous les autres groupes). De plus, le pourcentage de liaison associée aux sites agonistes de haute affinité était plus grand dans le cortex frontal des échantillons MA et DIM ($p \leq 0.05$), comparés aux MIXTE et aux témoins, et dans le cortex temporal du groupe MA comparé à tous les autres groupes ($p \leq 0.05$). Ces résultats nous permettent de croire qu'il y a une régulation à la hausse spécifiquement régionale des récepteurs cérébraux muscariniques dans la démence, spécialement dans la MA.

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Although several biochemical parameters in brains of demented patients differ from those of age-matched controls, only the decrement in choline acetyltransferase (ChAT) has been reported consistently and correlated with the loss of functional capacity.[1,2,3] In the present study, ChAT activity and muscarinic cholinergic binding were measured in brain samples obtained during the postmortem examinations of patients who died with dementia and from non-neurological controls. Ten brain regions were examined in brains from 26 patients with dementia of the Alzheimer type (AD), 4 patients with multi-infarct dementia (MID), 13 patients with morphological evidence of both AD-and MID-like lesions (MIXED dementia), and from 16 non-neurological control individuals.

Materials and Methods

Brains were obtained with a 10-12 hour average postmortem delay. The clinical diagnosis of dementia was confirmed or modified by necropsy examination of the left hemisphere, as described previously.[4] Control subjects had no clinical or necropsy evidence of dementia and no history of neurological difficulties. The mean age (range) in years for the subjects was as follows: control, 62 (32-75); AD, 76 (60-89); MID, 83 (77-97); MIXED, 83 (79-85). With the exception of 5 control and 2 MIXED subjects, the subjects were either before or at the time of their deaths receiving a variety of drugs, many of which had central anticholinergic properties.

From each individual, tissue for biochemical analysis was removed from a total of 10 frontal, temporal and hippocampal regions of the right hemisphere. Immediately after the dissection, the brain samples were either rapidly deep frozen to −70°C or sonicated in 20 volumes (wt/vol) of glass distilled water, then deep frozen to −70°C until the time of assay. Preliminary studies in our laboratory showed that the handling and storage of tissue in this manner did not significantly alter the biochemical findings (data not shown).

ChAT activity was determined according to the method of Bull and Oderfeld-Nowak.[5] Muscarinic binding was determined with [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) as the ligand and carbamylcholine as a displacing agent, using the "abbreviated assay" method of McKinney and Coyle.[6] This procedure can be used to estimate total muscarinic binding (B_{max}) and the percentage of B_{max} associated with the high affinity agonist (carbamylcholine) binding site ($\%B_{Hi}$) from the Law of Mass Action provided that reasonable estimates of the dissociation constants for [^{3}H]QNB (K_{QNB}) and the affinities of the agonist for high and lower affinity binding sites ($K_{Hi\text{-}Carb}$ and $K_{Lo\text{-}Carb}$, respectively) are known. Estimates for these parameters have been presented previously.[7] The concentrations of [^{3}H]QNB and carbamylcholine were fixed at 1.75 nM and 20 μM, respectively. ChAT activity and total specific [^{3}H]QNB binding (B_{max}) were reported in terms of supernatant protein, which was assayed by the method of Lowry et al.[8]

Data on [^{3}H]QNB binding in individual brain regions were combined to give pooled values for larger brain areas before the initial statistical analysis. Data from brain areas where differences were expected *a priori* were subjected to separate analyses for the individual component regions. Differences among diagnostic groups were assessed separately by region using an analysis of covariance,[9] controlling for possible effects associated with age, sex or the batch in which the tissue was transported from London, Ontario to Baltimore, Maryland and analyzed. Multiple comparisons were made using a Newman-Keuls procedure.[10]

Results

In comparison with non-demented control values, ChAT activity was reduced in each of the brain regions from demented patients (Table 1). While there were no statistical differences between the dementia groups, ChAT activity was consistently lowest in AD brains.

Figure 1 shows B_{max} values for each diagnostic group in each of the three brain areas. There were no significant intergroup differences for any of the three areas, although binding was generally lower in the temporal and frontal areas and higher in the hippocampal area of demented brains than in the same areas of the control group. However, statistical differences were observed in two of the regions sampled from the hippocampal area, the hippocampal endplate and H_1-subicular regions (Table 2). In the hippocampal endplate, AD B_{max} values were over two times greater than in control brains ($p \leq 0.01$), but did not differ significantly from those of MID or MIXED brains. In the H_1-subicular region, B_{max} values from AD brains were between 18% and 49% greater than corresponding values from the other three diagnostic groups ($p \leq 0.05$).

The percentage of binding associated with high affinity agonist sites in each of three areas is shown in Table 3. There were significant group differences in $\%B_{Hi}$ in the temporal (AD and MID > control and MIXED; $p \leq 0.05$) and frontal (AD > all other groups; $p \leq 0.05$) cortices, but no significant difference in $\%B_{Hi}$ in the hippocampal area. Comparison of the values for $\%B_{Hi}$ measured in component regions of the temporal and frontal areas revealed no significant differences (data not shown).

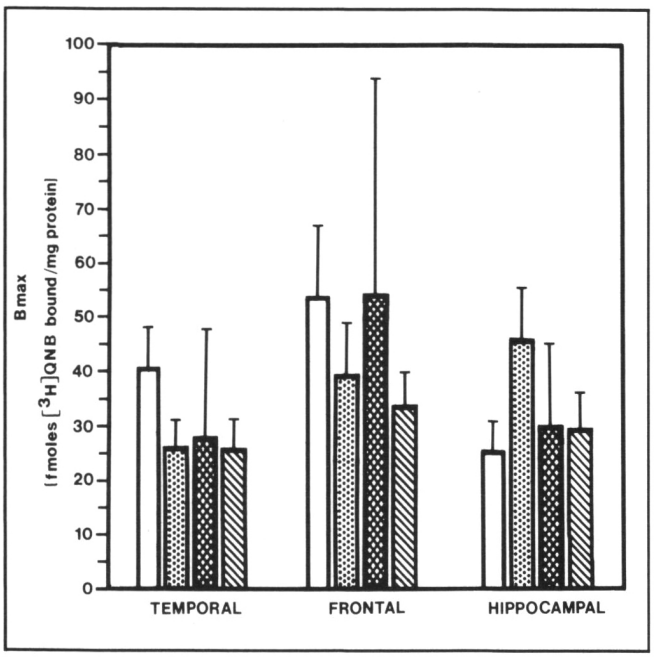

Figure 1 — Total muscarinic binding (B_{max}) in three brain areas from non-neurological control subjects (open bars) and patients who died with AD (stippled bars), MID (crosshatched bars), or MIXED dementia (diagonal bars). The number of subjects per group is shown at the bottom of each bar. Error bars indicate SEM.

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Table 1: Choline acetyltransferase activity in regions of brain taken from patients who died with a dementia and from control subjects

	Control	AD	MID	MIXED
Temporal Regions				
Superior Temporal Gyrus	12.10 ± 1.78 (16)	3.81 ± 0.52 (26)	5.00 ± 1.32 (4)	6.68 ± 1.98 (13)
Middle Temporal Gyrus	10.96 ± 1.21 (16)	3.22 ± 0.41 (26)	5.23 ± 1.27 (4)	4.86 ± 1.28 (13)
Inferior Temporal Gyrus	9.53 ± 1.00 (16)	3.29 ± 0.44 (25)	4.14 ± 1.17 (4)	4.60 ± 1.11 (13)
Frontal Regions				
Prefrontal Gyrus	9.06 ± 0.83 (16)	3.48 ± 0.42 (26)	5.37 ± 0.70 (4)	5.11 ± 0.76 (13)
Second Frontal Gyrus	9.36 ± 0.94 (16)	3.40 ± 0.47 (26)	6.18 ± 1.39 (4)	5.40 ± 1.17 (13)
Precentral Gyrus	8.83 ± 1.38 (16)	3.14 ± 0.45 (26)	3.40 ± 0.38 (4)	5.33 ± 0.95 (13)
Hippocampal Regions				
Hippocampal Endplate	30.29 ± 4.17 (15)	6.66 ± 1.41 (24)	16.16 ± 5.76 (4)	10.52 ± 3.90 (10)
H_2-Area	31.80 ± 5.11 (13)	10.15 ± 1.10 (23)	13.69 ± 5.22 (4)	17.75 ± 5.71 (10)
H_1-Subiculum	20.76 ± 2.70 (14)	10.63 ± 3.67 (24)	5.89 ± 3.49 (4)	8.07 ± 2.06 (11)
Presubiculum	14.18 ± 2.96 (16)	5.16 ± 0.52 (24)	5.60 ± 1.03 (4)	8.60 ± 1.75 (13)

Values are means ± S.E.M. (n) expressed as nmoles acetylcholine produced per hour per mg protein.
Values for all regions of demented brains were significantly lower than those of controls ($p \leq 0.05$). AD, dementia of the Alzheimer's type; MID, Multi-infact dementia; MIXED, mixed (AD and MID) dementia.

Table 2: Total muscarinic binding (B_{max}) in hippocampal brain regions from demented and control subjects

Region	Control	AD	MIXED	MID
Hippocampal Endplate	19.3 ± 5 (15)	59.8 ± 39 (23)*	26.1 ± 8 (10)	38.6 ± 30 (4)
H_2-Region	46.1 ± 4 (13)	28.1 ± 5 (22)	23.5 ± 3 (10)	23.9 ± 8 (4)
H_1-subiculum	37.2 ± 22 (14)	87.0 ± 15 (23)**	23.8 ± 2 (11)	24.5 ± 5 (4)
Presubiculum	21.7 ± 6 (16)	41.2 ± 16 (23)	21.5 ± 8 (13)	36.6 ± 24 (4)

Values are means ± S.E.M. (n) expressed as fmoles [^{3}H]QNB bound/mg protein; AD, dementia of the Alzheimer's type; MID, multi-infarct dementia; MIXED, mixed (AD and MID) dementia.

* Significant from control, $p \leq 0.01$.

** Significant from other diagnostic groups, $p \leq 0.05$.

Table 3: Percentage of total muscarinic binding associated with high affinity carbamylcholine binding site in three brain areas from demented and control subjects

	%B_{Hi}			
	Control	AD	MID	MIXED
Frontal Area	46.41 ± 3.3 (16)	54.3 ± 2.86 (24)**	48.31 ± 5.1 (4)	45.07 ± 6.87 (13)
Temporal Area	37.37 ± 3.3 (16)	54.54 ± 2.7 (25)**	57.53 ± 6.3 (4)**	43.07 ± 4.9 (13)
Hippocampal Area	51.63 ± 2.1 (13)	42.26 ± 3.2 (20)	40.26 ± 2.9 (2)	49.59 ± 6.8 (9)

Values are means ± S.E.M. (n). AD, dementia of the Alzheimer's type; MID, multi-infarct dementia; MIXED, mixed (AD and MID) dementia.

* Significantly different from all other groups, $p \leq 0.05$.

** Significantly different from control and MIXED groups, $p \leq 0.05$.

Discussion

The present findings confirm the consistent and robust loss of ChAT activity in frontal, temporal and hippocampal brain regions with AD.[1,2,3] New information is provided regarding the loss of ChAT activity in brain regions of individuals who died with MID or MIXED dementia and in the regional losses of ChAT activity associated with AD. Little attention has been focussed previously on the cholinergic involvement in non-AD dementias primarily because MID and MIXED are more varied than AD and thereby less likely to provide consistent changes. The present study demonstrates that like AD, MID and MIXED are associated with ChAT decrements in the frontal, temporal and hippocampal areas. It is now generally accepted that many symptoms of dementia are related to the decline in ChAT. Supporting this theory are observations that the loss of ChAT is correlated with the decrement of intellectual function in AD.[4,11,12,13] Further study is required to elucidate whether ChAT losses in MID or MIXED correlate with decrements of intellectual function.

Our investigation of muscarinic binding in dementia suggests that while there are several regionally specific changes associated with dementia, they are not as widespread or consistent as the well documented presynaptic changes. While Davies and Verth[14] and more recently Jenni-Eiermann et al[15] have reported a trend for increased muscarinic binding in dementia, this is the first report of a significantly higher density of muscarinic binding in brain regions from demented patients. We report a significantly higher estimate of B_{max} in the hippocampal endplate

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and H_1-subiculum regions and a greater proportion of binding associated with high affinity agonist sites in the temporal and frontal areas of AD and MID brains compared to control and MIXED groups. Contrasting with the present findings are at least ten separate reports of no change in muscarinic binding density in brain areas of demented patients compared to control;[2,14,15,16,17,18,19,20] and six reports of decreased muscarinic binding in regions of brains from demented patients compared to control.[15,21,22,23,24,25]

Pharmacological manipulation of cholinergic tone might result in receptor changes similar to those reported. Chronic blockade with muscarinic antagonists increases receptor density and alters the relative concentration of receptor subtypes.[6,26,27] Of the 58 subjects in the present study, all but seven had histories of recent exposure to centrally acting anticholinergics. Therefore, the receptor changes noted here may reflect drug treatments superimposed on the primary effects of the disease.

The reduced activity of ChAT in each of the regions tested is consistent with a presynaptic loss while the increased density of muscarinic binding sites in two regions and the elevation in $\%B_{Hi}$ in the temporal and frontal areas suggest postsynaptic changes. The changes in muscarinic binding are in keeping with a compensatory receptor upregulation in response to presynaptic losses. A similar involvement of brain cholinergic systems was suggested by Nordberg et al,[16] who observed that although mean densities of muscarinic binding sites did not differ significantly comparing AD with control hippocampal samples, the specific activity of ChAT was negatively correlated with muscarinic binding. The relationship between presynaptic cholinergic activity and muscarinic receptor density and subtype remains controversial. Data from animal studies concerning this problem suggest regional variability in the modulation of receptors to cholinergic input. For example, while denervation did not affect receptor density in sympathetic ganglia,[28] the iris,[29] or the hippocampus;[26] denervation by ibotenic acid injected into the nucleus basalis of Meynert resulted in an increased proportion of high affinity binding sites in the neocortex.[6]

There is evidence that similar regional variability in responsiveness exists in human brains as well. Reisine et al[30] and Ruberg et al[31] examined muscarinic binding and ChAT activity in patients who died with Parkinson's disease. In the study by Reisine et al,[30] ChAT activity was reduced both in the putamen and globus pallidus, while [^{3}H]QNB binding was significantly different (increased) only in the putamen. Similarly, Ruberg et al[31] reported elevated [^{3}H]QNB binding in the frontal cortex with no change in the caudate nucleus, although ChAT activity in both regions was significantly reduced compared to control. Both reports support the concept of regionally specific sensitivity to modulation by the presynaptic deficit of ChAT.

Inherent within this context is the importance of selection of regions to be examined, as demonstrated by the present study. Examination of the hippocampal area, all hippocampal regions combined, revealed only a small nonsignificant elevation in receptor binding. However, when the individual regions of the hippocampal area were examined, significant differences became evident in two of the four regions surveyed. It is possible that other investigators have not reported receptor changes similar to what is reported here because of differences in the dissection of the regions examined rather than methodological issues or differences in subject populations, although these factors cannot be eliminated from consideration.

It is of considerable interest that Hyman et al[32] noted a specific pattern of pathology of the CA1 and subiculum (the H_1-subiculum in the present study) in AD. This area receives afferents from numerous intrinsic hippocampal circuits as well as sending output to other parts of the brain. Such a specific cellular loss could contribute greatly to the functional deficits associated with the hippocampus in AD. In light of the importance of this region to overall hippocampal function,[33] compensatory upregulation of receptors to reduced input would be of considerable value in maintaining hippocampal function.

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