Neurotransmitter and Receptor Deficits in Senile Dementia of the Alzheimer Type

R. Quirion, J.C. Martel, Y. Robitaille, P. Etienne, P. Wood, N.P.V. Nair and S. Gauthier

ABSTRACT: Multiple neurotransmitter systems are affected in senile dementia of the Alzheimer’s type (SDAT). Among them, acetylcholine has been most studied. It is now well accepted that the activity of the enzyme, choline acetyltransferase (ChAT) is much decreased in various brain regions including the frontal and temporal cortices, hippocampus and nucleus basalis of Meynert (nbm) in SDAT. Cortical M₂-muscarinic and nicotinic cholinergic receptors are also decreased but only in a certain proportion (30-40%) of SDAT patients. For other systems, it appears that cortical serotonin (5-HT)-type 2 receptor binding sites are decreased in SDAT. This diminution in 5-HT₂ receptors correlates well with the decreased levels of somatostatin-like immunoreactive materials found in the cortex of SDAT patients. Cortical somatostatin receptor binding sites are decreased in about one third of SDAT patients. Finally, neuropeptide Y and neuropeptide Y receptor binding sites are distributed in areas enriched in cholinergic cell bodies and nerve fiber terminals and it would be of interest to determine possible involvement of this peptide in SDAT. Thus, it appears that multi-drug clinical trials should be considered for the treatment of SDAT.

NEUROPATHOLOGICALLY, SENILE DEMENTA OF THE ALZHEIMER TYPE (SDAT) is characterized by higher than normal densities of “senile” plaques and neurofibrillary tangles in various cortical regions, and by marked cell losses in the nucleus basalis of Meynert (nbm).¹⁻⁶ The neurochemical identity of these cell bodies projecting to the neocortex has been shown to be mostly cholinergic.⁷⁻¹² Interestingly, the presence in cortical plaques and tangles of cholinergic markers¹⁴ as well as other neurotransmitters and neuropeptides¹⁵⁻²⁰ has been recently demonstrated. However, it remains to be demonstrated if cell losses in the nbm trigger cortical damages or if multiple cortical insults retrogradely affect cell bodies in various subcortical nuclei.

THE DEGENERATION OF CHOLINERGIC CELL BODIES IN THE NBM HAS GENERATED MUCH INTEREST OVER THE LAST TEN YEARS. Various groups have clearly shown that cortical and subcortical cholinergic markers are markedly affected in SDAT, especially in the nbm-cortical pathway and hippocampus.²⁵⁻⁶,¹⁰,²¹⁻²⁸ Thus, a great majority of neurochemical studies has focussed on the characterization of cholinergic deficits in SDAT. Similarly, clinical trials in SDAT patients have concentrated, without much success, on using cholinergic-related drugs (for a recent review, see ²⁹).

However, recent data have clearly demonstrated that other neurotransmitter systems are also affected in SDAT including noradrenaline,³⁰⁻³⁶ serotonin (5-HT),³⁰,³⁵⁻⁴² somatostatin,³⁶,⁴³⁻⁵³

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glutamate,36,52 GABA27,36 and possibly Neuropeptide Y.53 In THE CANADIAN JOURNAL OF NEUROLOGICAL SCIENCES cholinergic, serotonergic and somatostatin systems are affected the total population of muscarinic receptors have been reported.62 It is also of interest from etiological and therapeutic perspectives. Assay.54 Moreover, it is usually assumed that changes in cortical ChAT activity closely reflect the status of the cholinergic synapse and correlate with the severity of SDAT.6 Consequently, it could also be suggestive of defects in the anterograde axonal transport of ChAT in SDAT.28 In any case, these data clearly demonstrate major losses in the capacity of synthesizing acetylcholine in brain of SDAT patients.55,56 Possible alterations in cholinergic receptor binding sites are also of interest from etiological and therapeutic perspectives. Up to date, most studies have concentrated on muscarinic receptor binding sites in SDAT. Early on, major decreases in the total population of muscarinic receptors have been reported.62 However, most subsequent studies did not confirm this finding and suggested that muscarinic receptor binding sites were not significantly altered in SDAT.35-38,52,63-65 Recent data could explain these discrepancies. It has recently been demonstrated that muscarinic receptors do not represent an homogenous population of sites but can be divided into two sub-types of receptors, M1 and M2.66,67 M1 receptors are mostly excitatory, insensitive to N-ethylnaleimide, independent of the presence of cyclic nucleotides and densely located in cortex, hippocampus and striatum.66,67 Preferential ligands include pirenzepine and at low concentrations, quinuclidinyl benzylate (QNB).38,66,67 M2 receptor binding sites are generally inhibitory via the blockade of adenylyl cyclase, are sensitive to N-ethylnaleimide and found in cholinergic nerves, striatum, cortex, superior colliculus, brainstem, thalamus and various peripheral tissues.66,67 Preferential ligands include acetylcholine itself and oxotremorine-M.59,66,67 An example of the distribution of [3H]acetylcholine-M2 binding sites in human brain is shown in Figure 1. Interestingly, it has recently been shown that the density of M2 muscarinic receptor sub-type is selectively decreased in SDAT.68 It was also suggested that this decrease was related to the presynaptic localization of M2 receptor binding sites on cholinergic nerve terminals.68 We have performed similar experiments and found that between 30-50% of SDAT patients showed significant decreases in the total population of muscarinic receptor binding sites (Table 1, [3H]QNB binding). However, the density of M1 sites (labelled with [3H]pirenzepine;58) was normal in all SDAT patients while that of M2 sites (labelled with [3H]acetylcholine;59) was decreased in about one third of the patients (Table 1). This suggests that alterations in the total population of muscarinic binding sites (measured with [3H]QNB) probably reflect changes in the density of the M2 receptor sub-type. Moreover, it appears that the percentage of M2 receptor losses are highly variable between SDAT patients (Table 1, 69). Thus, it seems unlikely that alterations in the density of muscarinic receptors would be

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<td>71.7±4.7</td>
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<td>686</td>
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<td>75</td>
<td>24.1±2.1</td>
<td>1.1±0.3</td>
<td>76.6±6.2</td>
<td>952</td>
<td>714</td>
<td>84</td>
<td>20</td>
<td>12</td>
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<tr>
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<td>57.9±6.4</td>
<td>543</td>
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<td>82</td>
<td>22.0±2.1</td>
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<td>832</td>
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<td>21</td>
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<td>58.1±6.2</td>
<td>1036</td>
<td>614</td>
<td>91</td>
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<td>4.1±0.2</td>
<td>45.8±2.9</td>
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<tr>
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<td>5.6±1.4</td>
<td>85.9±21.5</td>
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<td>782</td>
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Data represent means ± S.E.M. (for ChAT activity) of 3-6 determinations. All binding area are derived from full saturation analysis and represent maximal binding capacity (Bmax) in fmol/mg protein. All clinical and neuropathological data on these brain tissues have been reported before.28 ChAT activity has been determined as described before.52-61
Whitehouse et al have just reported that [3H] acetylcholine nicotinic receptor binding sites were markedly decreased in various cortical areas in SDAT. Previous studies were not as conclusive possibly due to the use of inappropriate ligands (α-bungarotoxin). As shown in Table 1, we also found that cortical nicotinic receptor binding sites are decreased in a certain proportion of SDAT patients. [3H]nicotine binding was significantly decreased only in patients that had marked alterations in both nbm and cortical ChAT activities (Table 1). This could indicate that nicotinic receptor binding sites are preferentially located on cholinergic neurons in brain. Further investigations on nicotinic receptors in SDAT are certainly warranted.

It is also possible to monitor the integrity of the cholinergic nerve terminals by studying the activity of the high-affinity choline uptake (HACU) system located presynaptically. Already, it has been shown that its activity is markedly decreased in the cortex of patients dying from SDAT. It should also be possible to use [3H]hemicholinium-3 (HC-3), a selective blocker of the HACU, to study possible modifications of this uptake mechanism in SDAT. This ligand has recently been used in rat brain tissues and the distribution of [3H]HC-3 binding sites correlates very highly with the localization of cholinergic cell bodies and nerve fiber terminals. Moreover, a selective lesion of the cholinergic nbm-cortical pathway decreased [3H]HC-3 binding in the cortex (Figure 2; ). However, it has been very difficult to obtain reliable results with [3H]HC-3 in human brain, either in post-mortem or fresh biopsied tissues (R. Quirion, unpublished results). Thus, it appears that other radiolabelled probes will have to be used to assess the status of the cholinergic presynaptic nerve terminals in SDAT. One of them could be AH-5183, a selective blocker of the vesicular transport of acetylcholine. [3H]AH-5183 has already been used to characterize these transporter sites in torpedo californica and rat brain. We are currently studying if it can be used in human brain tissues. If so, it would be of interest to determine if ChAT activity and [3H]AH-5183 binding are always affected in similar fashion in SDAT.

**Brain Serotonergic Markers in SDAT**

Beside the cholinergic system, much evidence has indicated possible involvement of the serotonergic (5-HT) innervation in the etiology of SDAT. Cell losses and the presence of tangles in the raphe nucleus, a region enriched in 5-HT cell bodies, have been reported. Consequently, major decreases in the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) have been observed in several brain regions including the frontal cortex, insula, cingulate cortex, amygdala, hippocampus and hypothalamus in SDAT. The specific 5-HT uptake mechanism could also be altered since reduced serotonin uptake and loss of imipramine binding have been reported. Moreover, 5-HT receptor binding sites are decreased in SDAT, especially the 5-HT 2 receptor sub-type. Marked decreases (up to 50%) in the density of 5-HT 2 binding sites have been found, especially in the hippocampus, frontal and temporal cortices. In our study, we observed marked losses in 5-HT 2 binding sites in the temporal cortex of patients dying from SDAT (Table 2). Thus, unlike the various cholinergic receptor sub-types, 5-HT 2 receptors seem to be decreased in the brain of all SDAT patients. Interestingly, there is also some evidence that alterations in 5-HT 2 binding sites are more pronounced in the early onset-type of SDAT and it is possible...
that the decrease in 5-HT2 binding sites could be related to the localization of these sites on cholinergic nerve terminals in the nbm-cortex pathway. Other 5-HT receptor sub-types of the 5-HT1 group (5-HT1A, 5-HT1B, 5-HT1C) are possibly slightly decreased in SDAT. However, a recent study has not been able to confirm this finding.36

In summary, it seems clear that 5-HT, its metabolites and especially 5-HT2 receptor sub-types are decreased in SDAT. Thus, therapeutic approaches using 5-HT related drugs alone or in combination with other (cholinergic) drugs should be carefully considered.

Somatostatin in SDAT

Among the various neuropeptides studied for possible alterations of their levels in SDAT, somatostatin (SS) is certainly the only one that consistently showed marked decreases. Various reports have clearly demonstrated that SS levels are much decreased in SDAT, especially in the temporal and frontal cortices, hippocampus and cerebrospinal fluid.44-51 We have also obtained similar data in our series of SDAT patients (Table 3). Interestingly, the decrease in SS-like immunoreactivity correlates well with 5-HT2 receptor losses35 suggesting possible association between these two "markers" in cortical brain regions.

A recent report has also suggested that SS receptor binding sites could be markedly decreased in cortical areas in SDAT.51 However, our data suggest that SS receptor binding sites are diminished only in a certain proportion of SDAT patients (Table 3) and further studies will be necessary to more precisely determine the exact status of SS receptors in SDAT.

In any case, it already suggests that somatostatin (or analogues) replacement therapies should be considered, at least for the sub-population of SDAT patients in which SS receptors are not altered. In that regard, the recent demonstration that SS delays extinction and reverses electroconvulsive shock-induced amnesia in rats is of great interest.98

Neuropeptide Y in SDAT

Neuropeptide Y (NPY) is one of the most highly concentrated peptides in the brain.89-91 High levels of NPY are especially found in the cortex, hippocampus and hypothalamus.89-91 Interestingly, NPY is co-localized with SS in various brain regions including the cortex, striatum and hippocampus. Very little is known on NPY receptor binding sites in brain tissues.92-94

Table 3: Somatostatin (SS)-like immunoreactivity and somatostatin receptor binding sites in temporal cortex of patients dying from senile dementia of the Alzheimer type

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at Death (Years)</th>
<th>ChAT Activity (nmol/mg protein/h)</th>
<th>SS-IR (ng/mg protein)</th>
<th>[125I] SS-28 binding (fmol/mg protein)</th>
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<tbody>
<tr>
<td>Alzheimer’s Disease</td>
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<tr>
<td>Early Onset</td>
<td></td>
<td>0.9±0.1</td>
<td>0.24</td>
<td>48</td>
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<tr>
<td>Late Onset</td>
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<td>0.8±0.3</td>
<td>0.36</td>
<td>71</td>
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<tr>
<td>Control</td>
<td></td>
<td>1.1±0.3</td>
<td>0.27</td>
<td>60</td>
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For ChAT activity, data represent means ± S.E.M. of 3-6 determinations. Binding data are derived from full saturation analysis and represent maximal binding capacity (Bmax). All clinical and neuropathological data have been reported before. ChAT activity has been determined as described before. [125I] ketanserin binding has been performed as described before.35

Table 2: Serotonin-type 2 (5-HT2) receptor binding sites in temporal cortex of patients dying from senile dementia of the Alzheimer type

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at Death (Years)</th>
<th>ChAT Activity (nmol/mg protein/h)</th>
<th>[3H] Ketanserin Binding (fmol/mg protein)</th>
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<tr>
<td>Alzheimer’s Disease</td>
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<tr>
<td>Early Onset</td>
<td></td>
<td>0.9±0.1</td>
<td>54</td>
</tr>
<tr>
<td>Late Onset</td>
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<td>0.8±0.3</td>
<td>43</td>
</tr>
<tr>
<td>Control</td>
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<td>1.1±0.3</td>
<td>39</td>
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</tbody>
</table>

For ChAT activity, data represent means ± S.E.M. of 3-6 determinations. Binding data are derived from full saturation analysis and represent maximal binding capacity (Bmax). All clinical and neuropathological data have been reported before. ChAT activity has been determined as described before.35

Table 1: Neuropeptide Y receptor binding sites in temporal cortex of patients dying from senile dementia of the Alzheimer type

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at Death (Years)</th>
<th>Neuropeptide Y (fmol/mg protein)</th>
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<td>Early Onset</td>
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<td>114</td>
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<td>Late Onset</td>
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<td>127</td>
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<tr>
<td>Control</td>
<td></td>
<td>114</td>
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</tbody>
</table>

For ChAT activity, data represent means ± S.E.M. of 3-6 determinations. Binding data are derived from full saturation analysis and represent maximal binding capacity (Bmax). All clinical and neuropathological data have been reported before. ChAT activity has been determined as described before.35
but we have recently described the autoradiographic distribution of NPY binding sites in rat brain. High densities of sites are present in cortex, hippocampus, thalamus and septum (Figure 3). Thus, high levels of NPY and NPY receptor binding sites are also been demonstrated. Studies on NPY and NPY receptors in SDAT are of interest, especially since this peptide is often co-localized with SS and its receptors are present in areas enriched with cholinergic innervation.

CONCLUSIONS AND PERSPECTIVES

In summary, multiple neurotransmitter systems are affected in SDAT. The cholinergic innervation is certainly much decreased, especially in the nbm, temporal cortex and hippocampus. Of the various markers used to monitor the cholinergic synapse, it was found that cortical ChAT activity is much decreased in SDAT. The high affinity choline uptake and the acetylcholine storage system are also most likely decreased in all cases. In terms of receptors, it seems that cortical muscarinic-M2 receptors and nicotinic receptors are significantly decreased in a sub-population of SDAT patients. M1 receptors are not affected. Thus, clinical treatments with cholinergic drugs could potentially be beneficial if they can reach remaining brain receptor sites. The intraventricular bethanechol infusions precisely attempt to examine this issue.

The serotonergic system is also affected in SDAT. Lower than normal levels of 5-HT and 5-HIAA are found in various cortical brain regions in certain patients while 5-HT2 receptors are markedly decreased in most, if not all, SDAT cases. Thus replacement therapies with potent 5-HT2-related drugs should be considered possibly in combination with cholinergic drugs. This could be most relevant especially since it has been recently shown that the combination of cholinergic and serotonergic agonists was more effective than either drug alone in restoring learning deficits in animals.

Somatostatin-like immunoreactivity is certainly much decreased in various cortical regions of brain SDAT patients while its receptors could be diminished in a certain percentage of SDAT patients. Thus, clinical trials with stable SS analogues should be planned, either in combination with other treatments (with cholinergic and/or 5-HT drugs) or alone. In any case, it appears that intracerebroventricular infusions will be the best approach, at least until the discovery of stable SS analogues capable of rapidly crossing the blood-brain barrier.

Finally, other neurotransmitter systems such as glutamate and catecholamines are most likely affected, at least in a certain proportion of SDAT patients. This markedly complicates the design of useful treatments of the disease and suggests multi-drug clinical trials. However, this will most likely generate a variety of side-effects that will be difficult to control. Thus, we believe that more global approaches would have to be envisioned in the future. For example, we are now focussing on the characterization of more general and basic deficits in the brain of SDAT patients. Thus, clinical trials with stable SS analogues should be planned, either in combination with other treatments (with cholinergic and/or 5-HT drugs) or alone. In any case, it appears that intracerebroventricular infusions will be the best approach, at least until the discovery of stable SS analogues capable of rapidly crossing the blood-brain barrier.

Acknowledgements

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