Neurotransmitter and Receptor Deficits in Senile Dementia of the Alzheimer Type

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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 M_2-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-HT_2 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.

RÉSUMÉ: Déficits au niveau des neurotransmetteurs et des récepteurs dans la démence sénile de type Alzheimer. Plusieurs systèmes de neurotransmetteurs sont atteints dans la démence de type Alzheimer (DSTA). Parmi eux l'acétylcholine a été le plus étudié. Il est maintenant reconnu que l'activité de l'enzyme choline acétyltransférase (ChAT) est très diminuée dans différentes régions du cerveau incluant le cortex frontal et temporal, l'hippocampe et le nucleus basalis de Meynert (nbM) dans la DSTA. Les récepteurs cholinergiques muscariniques de type M_2 et nicotiniques du cortex sont également diminués, mais seulement chez un certain nombre de patients (30-40%) atteints de DSTA. Pour ce qui est des autres système, il semble que les sites de liaison de type 2 pour la sérotonine (5-HT_2) sont diminués dans le cortex des patients atteints de DSTA. Cette diminution des récepteurs 5-HT_2 est en corrélation avec l'abaissement des niveaux de subtances immunoréactives semblables à la somatostatine que l'on trouve dans le cortex des patients atteints de DSTA. Les sites de liaison des récepteurs pour la somatostatine dans le cortex sont diminués chez le tiers des patients atteints de DSTA. Finalement le neuropeptide Y et les sites de liaison des récepteurs pour le neuropeptide Y sont répartis dans des zones riches en corps cellulaires et en terminaisons nerveuses cholinergiques et il serait intéressant d'évaluer l'implication possible de ce peptide dans la DSTA. Ainsi, il semble que des essais thérapeutiques portant sur l'administration simultanée de plusieurs médicaments devraient être envisagés dans le traitement de la DSTA.

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Neuropathologically, senile dementia 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.[7-13] Interestingly, the presence in cortical plaques and tangles of cholinergic markers[14] as well as other neurotransmitters and neuropeptides[15-20] 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.[2,5,6,8,10,21-28] 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,[36,43-51]

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Table 1: Status of various markers of the cholinergic synapse in senile dementia of the Alzheimer type

Case	Age at death (years)	ChAT activity (nmol/mg protein/hour) nbm	Temporal Cortex	Caudate Nucleus	[^{3}H] QNB Temporal Cortex	[^{3}H] Pirenzepine-M_1 Temporal Cortex	[^{3}H] Ach-M_2 Temporal Cortex	[^{3}H] Nicotine Temporal Cortex
Alzheimer's Disease								
Early Onset	68	7.4±0.2	0.9±0.1	52.6± 2.5	712	682	52	13
	60	25.7±5.1	0.8±0.3	71.7± 4.7	921	686	75	24
	75	24.1±2.1	1.1±0.3	76.6± 6.2	952	714	84	20
Late Onset	81	8.6±0.3	0.7±0.1	57.9± 6.4	543	524	31	12
	90	9.1±1.0	0.5±0.1	76.7± 2.7	602	615	40	10
	82	22.0±2.1	1.1±0.1	76.6± 6.2	991	832	79	21
Control	80	38.9±4.3	4.0±0.3	58.1± 6.2	1036	614	91	31
	82	25.1±1.2	4.1±0.2	45.8± 2.9	924	711	82	25
	61	26.0±1.5	3.3±0.2	41.3± 1.7	947	727	95	27
	63	45.0±9.7	5.0±1.2	84.0±19.9	1139	801	72	26
	81	67.0±9.9	—	62.8± 4.6	—	—	—	—
	84	25.9±4.4	5.6±1.4	85.9±21.5	814	709	79	25
	66	42.5±4.3	6.3±1.9	66.9±36.7	1087	747	84	30
	77	33.6±5.4	4.0±0.5	56.6±14.5	961	782	80	26

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 (B_{max}) 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 by Fonnum.[54] [^{3}H] QNB, [^{3}H] pirenzepine, [^{3}H] acetylcholine-M_2 and [^{3}H] nicotine binding have been determined as described before.[52-61]

glutamate,[36,52] GABA[27,36] and possibly Neuropeptide Y.[53] In this report, we present some evidence demonstrating that cholinergic, serotonergic and somatostatin systems are affected in SDAT. The possible involvement of another peptide, neuropeptide Y, is also discussed.

Brain Cholinergic Markers in SDAT

Numerous markers are available to study cholinergic neurons and the cholinergic synapse. Among them, the enzyme responsible for the acetylation of choline into acetylcholine, choline acetyltransferase (ChAT), has been most studied possibly due to its long post-mortem stability, reliability and ease to 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]

Multiple studies have clearly demonstrated that ChAT is markedly decreased in cortex (frontal, parietal and temporal), hippocampus and nbm in SDAT patients (early and late onset).[2,6,21-28,30,32,36,37,43,55] The decline in ChAT activity usually correlates with the high densities of senile plaques and neurofibrillary tangles present in cortex, and with cell loss in the nbm.[6,28] Interestingly, we have recently found that nbm ChAT activity was decreased only in certain SDAT patients while cortical ChAT activity was low in all cases (Table 1; [28]). This may indicate that decreased cortical ChAT activity precede and may trigger loss of enzymatic activity in the nbm. On the other hand, 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, M_1 and M_2.[66,67] M_1 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 radioligands include pirenzepine and at low concentrations, quinuclidinyl benzylate (QNB).[58,66,67] M_2 receptor binding sites are generally inhibitory via the blockade of adenylate 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 [^{3}H]acetylcholine-M_2 binding sites in human brain is shown in Figure 1.

Interestingly, it has recently been shown that the density of M_2 muscarinic receptor sub-type is selectively decreased in SDAT.[68] It was also suggested that this decrease was related to the presynaptic localization of M_2 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, [^{3}H]QNB binding). However, the density of M_1 sites (labelled with [^{3}H]pirenzepine;[58]) was normal in all SDAT patients while that of M_2 sites (labelled with [^{3}H] 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 [^{3}H]QNB) probably reflect changes in the density of the M_2 receptor sub-type. Moreover, it appears that the percentage of M_2 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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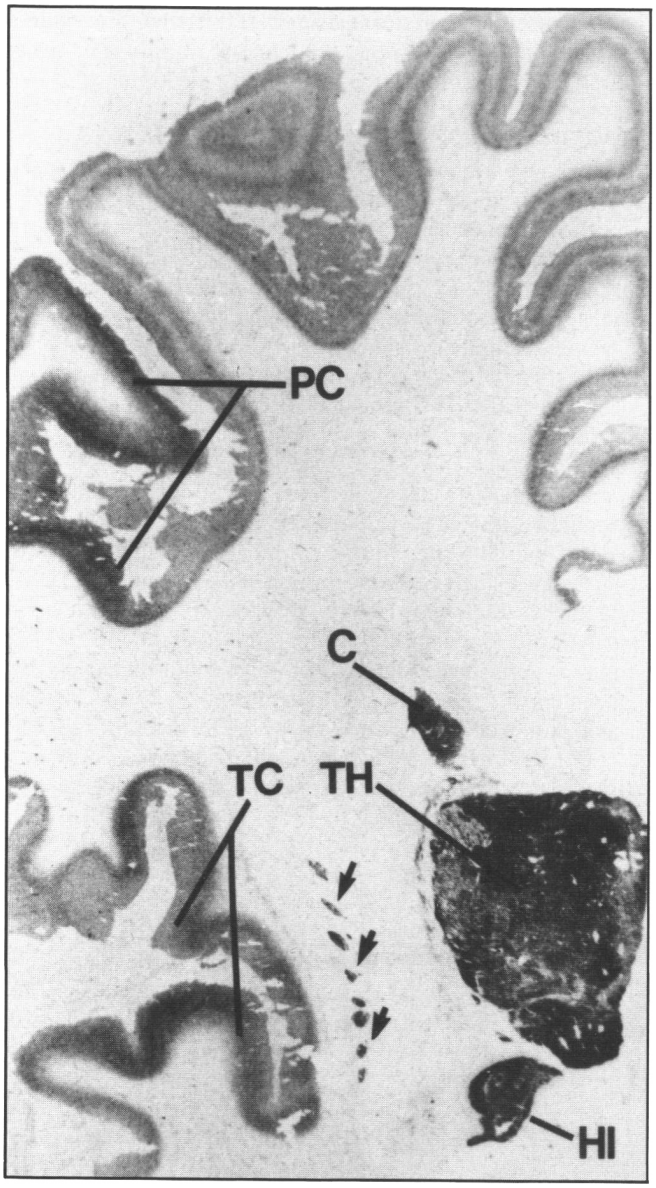

Figure 1 — Photomicrograph of the autoradiographic distribution of [^{3}H] acetylcholine-M2 (20 nM) binding sites in a representative coronal section of a control human brain at the level of the thalamus. High densities of sites are present in the thalamus, caudate and certain areas of the cortex. Section was incubated under conditions described before for rat brain.[59] Abbreviations used: C, caudate; HI, hippocampus; PC, parietal cortex; TC, temporal cortex; TH, thalamus and arrows, pontine grisea caudatolenticulares.

of major importance in the etiology of SDAT. Finally, the exact localization of the M_2 receptor sub-type remains to be established. Our results suggest that it is unlikely that all M_2 receptor binding sites are located on cholinergic cell bodies and nerve terminals. We found that the density of cortical M_2 binding sites was decreased in only 30-50% of SDAT patients while cortical ChAT activity was decreased in all cases (Table 1). Moreover, selective lesions of the nbm-cortical (kainic acid) and septo-hippocampal (fimbriaectomy) cholinergic pathways did not significantly alter [^{3}H]acetylcholine-M_2 binding in cortical and hippocampal areas in rat brain (Quirion and Richard, unpublished results). Thus, it would appear that M_2 receptors are not exclusively located on cholinergic cell bodies and nerve terminals (presynaptic) in rat and human brain tissues.

Whitehouse et al[60] have just reported that [^{3}H] 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).[70,71] As shown in Table 1, we also found that cortical nicotinic receptor binding sites are decreased in a certain proportion of SDAT patients. [^{3}H]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.[60] 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.[72,73] It should also be possible to use [^{3}H]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 [^{3}H]HC-3 binding sites correlates very highly with the localization of cholinergic cell bodies and nerve fiber terminals.[58,74-80] Moreover, a selective lesion of the cholinergic nbm-cortical pathway decreased [^{3}H]HC-3 binding in the cortex (Figure 2; [2,77,79]). However, it has been very difficult to obtain reliable results with [^{3}H]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.[72,82] [^{3}H]AH-5183 has already been used to characterize these transporter sites in torpedo californica[82] and rat brain.[83] 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 [^{3}H]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.[84,85] 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[30,33-37,42] in SDAT. The specific 5-HT uptake mechanism could also be altered since reduced serotonin uptake[37,86] and loss of imipramine binding[37] 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.[35,36,38-41] 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[35] and it is possible

that the decrease in 5-HT_2 binding sites could be related to the localization of these sites on cholinergic nerve terminals in the nbm-cortex pathway.[81] Other 5-HT receptor sub-types of the 5-HT_1 group (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}) are possibly slightly decreased in SDAT.[37] 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-HT_2 receptor sub-types are decreased in SDAT.

Table 2: Serotonin-type 2 (5-HT_2) receptor binding sites in temporal cortex of patients dying from senile dementia of the Alzheimer type

Case	Age at Death (Years)	ChAT Activity (nmol/mg protein/h) Temporal Cortex	[^{3}H] Ketanserin Binding (fmol/mg protein) Temporal Cortex
Alzheimer's Disease			
Early Onset	68	0.9±0.1	54
	60	0.8±0.3	43
	75	1.1±0.3	39
Late Onset	81	0.7±0.1	56
	90	0.5±0.1	50
	82	1.1±0.1	61
Control	80	4.0±0.3	91
	82	4.1±0.2	127
	61	3.3±0.2	114
	63	5.0±1.2	96
	81	—	—
	84	5.6±1.4	132
	66	6.3±1.9	95
	77	4.0±0.5	103

For ChAT activity, data represent means ± S.E.M. of 3-6 determinations.[28] Binding data are derived from full saturation analysis and represent maximal binding capacity (B_{max}). All clinical and neuropathological data have been reported before.[28] ChAT activity has been determined by Fonnum.[54] [^{3}H] ketanserin binding has been performed as described before.[81] ChAT activity is added for comparison.

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-HT_2 receptor losses[35] 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.[88]

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

Case	Age at Death (Years)	ChAT Activity (nmol/mg) protein/h) Temporal Cortex	SS-IR (ng/mg protein) Temporal Cortex	[^{125}I] SS-28 binding (fmol/mg protein) Temporal Cortex
Alzheimer's Disease				
Early Onset	68	0.9±0.1	0.24	48
	60	0.8±0.3	0.36	71
	75	1.1±0.3	0.27	60
Late Onset	81	0.7±0.1	0.31	32
	90	0.5±0.1	0.32	39
	82	1.1±0.1	0.30	66
Control	80	4.0±0.3	0.44	74
	82	4.1±0.2	0.63	62
	61	3.3±0.2	0.45	84
	63	5.0±1.2	0.59	69
	81	—	—	—
	84	5.6±1.4	0.60	70
	66	6.3±1.9	0.62	86
	77	4.0±0.5	0.54	61

For ChAT activity, data represent means ± S.E.M. at 3-6 determinations.[28] Binding data are derived from full saturation analysis and represent maximal binding capacity (B_{max}). All clinical and neuropathological data have been reported before.[28] ChAT activity has been determined as described by Fonnum.[54] SS-like immunoreactive materials and [^{125}I] Leu^8, D-trp^{22}, Tyr^{25} SS-28 binding have been assayed as described before.[48,87]

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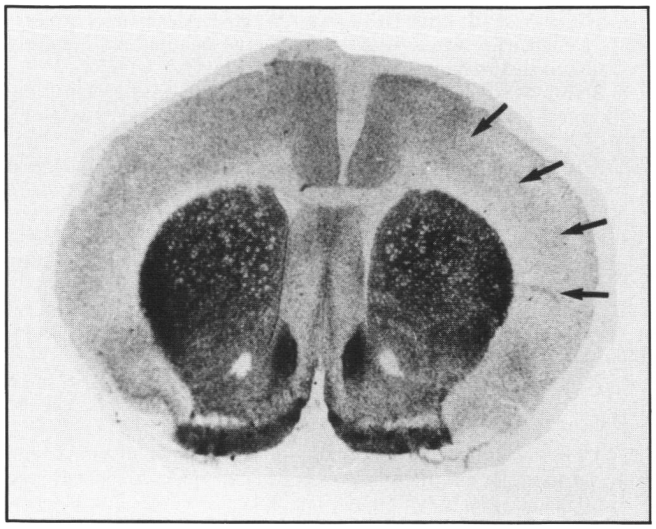

Figure 2 — Photomicrograph of the autoradiographic distribution of [^{3}H] hemicholinium-3 high affinity choline uptake sites in rat brain following a 7 day unilateral kainic acid lesion (right side) of the ventral pallidum-substantia innominata region. Note the decrease in [^{3}H]hemicholinium binding (10 nM) in cortex following lesion of the ventral pallidum area. Lesions and binding assays were performed as described before.[58,81]

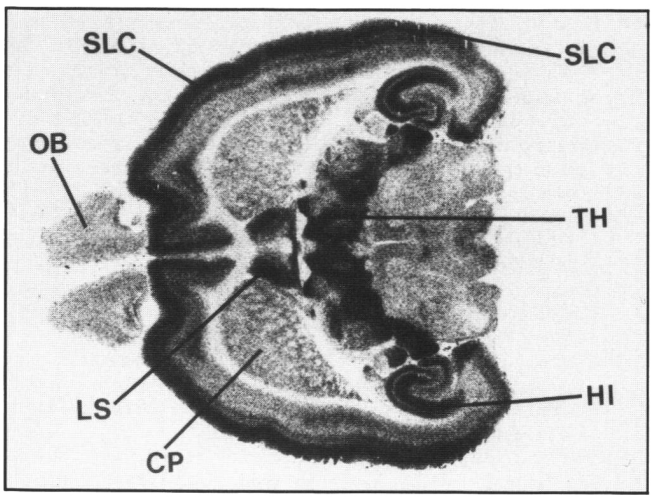

Figure 3 — Photomicrograph of the autoradiographic distribution [^{125}I] neuropeptide Y binding sites in a horizontal section of the rat brain. Brain sections were incubated and processed for [^{125}I]neuropeptide Y binding as described before.[95] Abbreviations used: CP, caudate-putamen; HI, hippocampus; LS, lateral septum; OB, olfactory bulb; SLC, superficial layers (I and II) of the cortex and TH, thalamus.

but we have recently described the autoradiographic distribution of NPY binding sites in rat brain.[95] 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 found in brain regions enriched with cholinergic innervation.

Only limited data have been reported on NPY-like immunoreactivity in SDAT patients.[96-98] Thus far, decreased levels,[98] modified laminar distribution[97] or normal levels[91,96] in NPY-like peptides have been found in cortex of SDAT patients. The presence of NPY-like immunoreactivity in neuritic plaques has also been demonstrated.[18] Studies on NPY receptor binding sites in SDAT brains have yet to be reported. Clearly, investigations 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-M_2 receptors and nicotinic receptors are significantly decreased in a sub-population of SDAT patients. M_1 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.[99,100]

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-HT_2 receptors are markedly decreased in most, if not all, SDAT cases. Thus replacement therapies with potent 5-HT_2-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, including possible alterations in protein translocation,[101] calcium mobilization[102] and neurotrophic factors.[103] We hope that it will be possible to develop more appropriate therapies based on drugs modulating these biochemical events.

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