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Cholinergic–serotonergic imbalance contributes to cognitive and behavioral symptoms in Alzheimer's disease

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Abstract

Neuropsychiatric symptoms seen in Alzheimer's disease (AD) are not simply a consequence of neurodegeneration, but probably result from differential neurotransmitter alterations, which some patients are more at risk of than others. Therefore, the hypothesis of this study is that an imbalance between the cholinergic and serotonergic systems is related to cognitive symptoms and psychological syndromes of dementia (BPSD) in patients with AD. Cholinergic and serotonergic functions were assessed in *post-mortem* frontal and temporal cortex from 22 AD patients who had been prospectively assessed with the Mini-Mental State examination (MMSE) for cognitive impairment and with the Present Behavioral Examination (PBE) for BPSD including aggressive behavior, overactivity, depression and psychosis. Not only cholinergic deficits, but also the cholinacetyltransferase/serotonin ratio significantly correlated with final MMSE score both in frontal and temporal cortex. In addition, decreases in cholinergic function correlated with the aggressive behavior factor, supporting a dual role for the cholinergic system in cognitive and non-cognitive disturbances associated to AD. The serotonergic system showed a significant correlation with overactivity and psychosis. The ratio of serotonin to acetylcholinesterase levels was also correlated with the psychotic factor at least in women. It is concluded that an imbalance between cholinergic–serotonergic systems may be responsible for the cognitive impairment associated to AD. Moreover, the major findings of this study are the relationships between neurochemical markers of both cholinergic and serotonergic systems and non-cognitive behavioral disturbances in patients with dementia.

Keywords: Cognitive decline; Behavioral and psychological syndromes of dementia (BPSD); Cholineacetyltransferase; 5-HT; Cortex; Human

1. Introduction

Disruption of basal forebrain cholinergic pathways and consequent cortical cholinergic denervation is one of the hallmarks of Alzheimer's disease (AD), together with histopathological changes such as neurofibrillary tangles and senile plaques. This cholinergic dysfunction in AD has been largely related to cognitive disturbances (Francis, Palmer, Snape, & Wilcock, 1999; Perry, Walker, Grace, & Perry, 1999). Besides cognitive symptoms, most patients with AD suffer from behavioral and psychological syndromes of dementia (BPSD; IPA, 1996), such as aggressive behavior, overactivity, depression or psychosis (Hope, Keene, Fairburn, McShane, & Jacoby, 1997) which are a major contributory factor to early institutionalisation of the patients (Levy, Cummings, & Kahn-Rose, 1999). Traditional treatments for BPSD are antipsychotic medications, benzodiazepines or anticonvulsant medications, which are not devoid of serious adverse effects, particularly in the elderly (Byerly et al., 2001). It is therefore important to understand the neurochemical basis of BPSD to allow the development of rational therapeutic approaches. In this sense, observations in which cholinergic replacement therapies resulted in behavioral improvements,

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independent from effects on cognition, in patients with AD (Paleacu, Mazeh, Mirecki, Even, & Barak, 2002) may have important clinical implications. However, it is conceivable that, due to the complexity and diversity of BPSD, more than one transmitter system may contribute to a particular behavioral syndrome; indeed, balance between pairs of neurotransmitters may be of particular importance, e.g. both reduced serotonergic and increased noradrenergic activities have been linked to aggressive behavior (Lancot, Herrmann, & Mazzota, 2001) and also a cholinergic–monoaminergic imbalance has been postulated in the pathogenesis of affective disorders (Cummings & Kaufer, 1996). Knowledge of imbalances in these systems offers the opportunity for rational treatment or prevention.

The involvement of the serotonergic system in higher cognitive processes such as memory and learning has been well described and there is evidence suggesting that changes in this neurotransmitter system occur in association with non-disease aging (Buhot, Martin, & Segu, 2000; Porter, Lunn, & O'Brien, 2003). In animal studies, a combined blockade of the cholinergic and serotonergic system contributes to a severe and widespread deterioration of adaptative behavior, condition that might be considered analogous to dementia in man (Vanderwolf, 1987). In addition, while a selective reduction in cholinergic transmission often produces only mild impairments in spatial memory and other behavioral tests, additional serotonergic blockade results in the appearance of severe behavioral deficits. Consequently, it has been argued that serotonin plays a role in the maintenance of behavioral capacities in the face of reduced cholinergic transmission (Dringenberg & Zalan, 1999). In AD, previous reports have shown extensive serotonergic denervation (Chen, Alder, & Bowen, 1996; Chen et al., 2000) although its clinical signification has been only partially defined. Serotonin (5-HT) has also been involved in many psychobiological functions such as aggression, depression, anxiety, or psychosis that are relevant to BPSD (Chen et al., 1996; Lancot et al., 2001). In this context, open label trials with selective serotonin reuptake inhibitors (SSRIs) have been associated with improvements in BPSD in a high proportion of patients (Parnetti, Amici, Lanari, & Gallai, 2001). In addition, serotonergic pathways are known to interact extensively with the cholinergic, noradrenergic, GABAergic and dopaminergic systems and therefore, serotonergic therapies may be used to manipulate other neurotransmitters systems to alleviate BPSD.

Different neurotransmitter systems, other than the serotonergic and cholinergic systems have been implicated in AD. Reductions in noradrenergic markers have been reported in AD patients (Matthews et al., 2002). Data on the dopaminergic system is not conclusive, and although some alterations have been described in AD patients (Storga, Vrecko, Birkmayer, & Reinnegger, 1996) dopamine levels are not affected in other studies (Minger et al., 2000; Witte et al., 1999). There is also considerable evidence of alterations in the glutamatergic system in AD and it has been described that reductions in glutamatergic markers correlate with the degree of dementia (for review see Danysx & Parsons, 2003; Francis, 2003).

In the present study, we have used clinical data and *post-mortem* brains collected as part of a prospective community-based study of behavior in dementia patients (Hope et al., 1997) to test the hypothesis that an imbalance between the cholinergic and serotonergic system contributes to both cognitive deficits and BPSD in AD. We have studied the state of the cholinergic and serotonergic neurotransmission in the cerebral cortex with respect to cognitive impairment and four behavioral syndromes (psychosis, overactivity, aggressive behavior and depression) previously identified in this patient group (Hope et al., 1997). We propose that cholinergic deficits alone may not be sufficient to cause the marked changes in cortical activity typical of AD. Cholinergic deficits may, however, make neural circuits more susceptible to additional neurochemical deficits and therefore, a compromised serotonergic function, in combination with a cholinergic deficit, may make an important contribution to the progression of the illness.

2. Methods

2.1. Drugs used

Superpure H_2O water (SpS, Romil), 0.22 μm filters (Millipore, UK). Acetylthiocholine iodide, sodium dodecyl sulphate, 5,5′-dithio-bis(2-nitrobenzoic) acid and eserine salicylate (Sigma-Aldrich Ltd, Germany), ^{14}C-acetyl CoA (Amersham, UK), ecoscint TM (National Diagnostics, UK). All other chemicals were purchased from Panreac, USA.

2.2. Patients and assessment of behavior

A total of 42 individuals were included in the study, 22 patients with clinical diagnosis of dementia and 20 elderly normal controls matched for age, gender, *post-mortem* delay and brain pH (Table 1). Those patients with dementia were an autopsied subset of subjects included in a prospective study of behavioral changes in clinically diagnosed as demented patients (Hope et al., 1997). Initially, all patients were living in community with a caregiver (usually spouse or daughter) who could inform accurately about day-to-day behavior. More than one informant was assigned to institutionalized patients when necessary. Drug histories were recorded

Table 1
Demographic features of patients

	Control	Alzheimer
Age (years)	74.75 ± 2.67	81.06 ± 1.60
Gender (man/woman)	11/9	11/13
Post-mortem delay (h)	39.28 ± 5.40	48.63 ± 6.30
pH	6.28 ± 0.16	6.44 ± 0.10

pH, standard chemical symbol, negative log of hydrogen ion concentration (control n = 19–20, Alzheimer n = 20–22). Values are mean ± S.E.M.

for all patients; 13 patients were taking major tranquilizers, and 8 were taking minor tranquilizers. None of the patients with AD received cholinomimetics. At entry to the study assessment and diagnoses were made using (CAMDEX) (Roth et al., 1986), DMS-III-R criteria (American Psychiatric Association, 1987) and NINCDS-ADRA criteria (McKhann, Drachman, & Folstein, 1984). Cognitive status was assessed at 4 monthly intervals using the Mini-Mental State Examination (MMSE) (Folstein & Folstein, 1975). Decline in MMSE was calculated as the difference between maximum scoring and the scoring obtained last time the patient was examined before death. Although the Mini-Mental State Examination (MMSE) (Folstein & Folstein, 1975) presents some limitations for the assessment of cognitive status in AD (Schmitt et al., 2002) it is a practical test to be used serially and routinely. Four behavioral and psychological syndromes were assessed using the Present Behavioral Examination (PBE) (Hope & Fairburn, 1992): depression, overactivity, psychosis and aggressive behavior (Hope et al., 1997). Briefly, the PBE is standardized, caregiver-based interview with high intra- and interrater reliavility that covers in detail the observable behavior and mental state of the patient. Questions to elucidate the caregiver answers were also included. Depression factor was the sum of four components: apparent sadness, gloomy thoughts, feeling like a failure and tearfulness. The overactivity factor consisted of the total of the highest ratings for walking and trailing + checking. The psychosis factor was the sum of scores for hallucinations, persecutory ideas and inappropriate anxiety. Three different aspects of aggressive behavior were used in this analysis. These were physical aggression, aggressive resistance and verbal aggression and the highest ratings were added together to give the aggressive behavior factor. Each component of the syndrome was scored from 0 to 2. A score of 0 meant that the behavior was absent. A score of 1 (mild) denoted that that particular type of behavior had occurred on up to half the days in the previous weeks. A rating of 2 (severe) meant that the behavior had occurred on half of the days or more. This gives a maximum score of 6 (or 8 for the depression factor). Factors were calculated from behavioral data for the last interview before death in order to correlate them with neurochemical data determined *post-mortem*.

2.3. Tissue samples and neuropathology

Informed consent had been obtained from relatives before for removal of brain tissue at death. At autopsy, brains were removed from 22 of the prospectively assessed cases with clinical diagnoses of dementia and from 20 elderly normal control cases. Blocks corresponding to frontal (Brodmann area 10, BA10) and temporal (Brodmann area 20, BA20) cortex were removed and stored at −80 °C until processed. All 22 patients were found to meet CERAD criteria (Mirra et al., 1991) for a diagnosis to AD. To partially mitigate the possible effects of cause of death on neurochemical determinations, brain pH was measured as an index of acidosis associated with terminal coma (Table 1). Brain pH is used as an indication of tissue quality in *post-mortem* research, with pH > 6.1 considered acceptable (Bahn et al., 2001; Lewis, 2002).

All subsequent analysis was performed blind to clinical information.

2.4. Acetylcholine (ACh) and choline measurements

ACh and choline measurements were performed following a method described by Takahashi, Ishimaru, Ikarashi, Kishi, and Maruyama (1997) with minor modifications. Briefly, cortical samples were homogenised in 20 volumes of 50 mM $HClO_4$ in Superpure H_2O water. Homogenates were centrifugated 10.000 g for 15 min. Supernants were filtrated by centrifugation 2000 *g* for 2 min with 0.22 μm filters. Concentrations of ACh and its metabolite, choline, were determined by high performance liquid chromatography (HPLC) with electrochemical detection. All samples were assayed in duplicate and results were expressed per mg of wet tissue.

2.5. Acetylcholinesterase (AChE) determination

AChE assay was performed using a colorimetric method reported by Wang et al. (1999) with minor modifications. Frontal or temporal cortex was homogenised in 39 volumes of 75 mM sodium phosphate buffer (pH 7.4). A mixture of 2 ml containing acetylthiocholine iodide, 0.5 ml sodium phosphate buffer (0.1 mM, pH 7.4) and 50 μl homogenate was incubated for 8 min. The reaction was then terminated by adding 0.5 ml 3% (w/v) sodium dodecyl sulphate followed by 0.5 ml 0.2% (w/v) 5,5′-dithio-bis(2-nitrobenxoic) acid to produce the yellow anion of 5-thio-2-nitro-benzoic-acid. The extent of colour production was measured spectrophometrically at 420 nm using Ultospec 3000 (Pharmacia Biotech). All samples were assayed in triplicate. Results were expressed as percentage of control values.

2.6. Cholinacetyltransferase (ChAT) activity

ChAT activity was performed using the method described by Fonnum (1975) with minor modifications. Frontal or temporal cortex was homogenised in 50 volumes of 0.87 mM EDTA contaning 0.1% Triton X-100 (pH 7.0). Duplicated samples of 10 μl from homogenates were used in the assay. The reaction mixture comprised the following: 87 nM EDTA (pH 7.4), 0.5 M NaH_2PO_4 (pH 7.4), 40 mM choline chloride 3 M NaCl, 2 nM eserine salicylate and 2 mM ^{14}C-acetyl CoA. Samples were incubated at 37 °C for 30 min. Water was used as sample blanks The reaction was terminated by adding 100 μl of cold water. The acetylcholine product was separated using Kalignost solution: 0.5% cold sodium tetraphenylborate in 15–85% acetonitrile–toluene. The supernatant was then transferred to scintillation cocktail (Ecoscint TM) and radioactivity was measured. Results were expressed as percentage of control values.

2.7. 5-HT and 5-HIAA measurements

5-HT and 5-HIAA concentrations were determined by HPLC with electrochemical detection as previously described by Perez-Otaño et al. (1991). Cortical samples were homogenised in 20 vol of extraction mixture (0.4 M percloric acid; 1 mM EDTA; 0.1% metabisulphitic acid and 50 ng/ml DHBA as internal standard). Homogenates were centrifugated at 32500 g for 20 min and 20 μl aliquots injected into the HPLC were analyzed. All samples were assayed in duplicate and results were expressed per mg of wet tissue.

2.8. Statistical analysis

Data were analysed by SPSS for Windows, release 11.0. Student's *t*-test was used in initial comparisons between control patients and patients with AD. The effects of demographic factors (age, *post-mortem* delay and brain pH) on neurochemical variables were determined by Pearson's product–moment correlations. Intercorrelation of neurochemical variables was also examined by Pearson's product–moment correlations. Spearman's rank correlation was also used for studies of the relationships between severity of dementia (MMSE score at last interview before death or MMSE decline) and neurochemical measures. Multiple regression analysis using "stepwise" method was used to investigate possible relationships between neurochemical variables and the behavioral syndromes. As individual patients may show more than one behavioral syndrome, multiple-regression indicates the strongest correlate (Minger et al., 2000).

3. Results

Demographic details of subjects are shown in Table 1. There were no significant differences in age, *post-mortem* delay or brain pH between the control and AD groups. In addition, no significant correlations between age, *post-mortem* delay or brain pH and any of the neurochemical variables studied in either the control or patients with dementia ($P > 0.05$) were found. However, they were included as covariates in order to avoid age or *post-mortem* delay interferences in subsequent analysis.

3.1. Neurochemical characterization of samples

All these results are summarised in Table 2.

3.1.1. Cholinergic system

Concentrations of both ACh and choline were significantly reduced in AD patients compared to controls in both BA10 and BA20. As shown in Table 2, ACh reductions reached 55% in BA10 and 60% in BA20.

Similarly, AChE activity in frontal and temporal cortex from AD patients was significantly lower than control patients. Reductions observed reached 30% in both cortical areas. ChAT activity was also reduced in BA10 (30%) and in BA20 (35%) from AD patients when compared to control values.

Individual concentrations of ACh were significantly correlated with both choline and AChE in BA10 from AD patients (ACh-choline $n = 22$; $r = 0.545^*$; $P = 0.009$; ACh-AChE $n = 21$; $r = 0.507^*$; $P = 0.019$). AChE and ChAT activities were also significantly correlated in BA10 ($n = 21$; $r = 0.507^*$; $P = 0.019$). In BA20, ACh levels were significantly correlated with and choline ($n = 19$; $r = 0.481^*$; $P = 0.037$) and AChE activity ($n = 19$; $r = 0.462^*$; $P < 0.045$).

3.1.2. Serotonergic system

Concentrations of both 5-HT and 5-HIAA were significantly reduced in BA10 and BA20 from AD patients. 5-HT reductions reached 42% in BA10 and 52% in BA20, whereas 5-HIAA levels were reduced in a 48% in BA10 and 55% in BA20 from AD patients.

5-HT and 5-HIAA levels were also correlated in BA10 ($n = 21$; $r = 0.478^*$; $P < 0.045$) and BA20 ($n = 21$; $r = 0.448^*$; $P < 0.042$).

Table 2
Neurochemical characterization of two cortical regions of patients with Alzheimer's disease and controls

	BA10		BA20	
	Control	Alzheimer	Control	Alzheimer
Cholinergic system				
ACh	0.36 ± 0.04	0.15 ± 0.02**	0.52 ± 0.08	0.22 ± 0.03**
Choline	377.55 ± 30.17	273.89 ± 25.07**	558.89 ± 29.36	257.55 ± 24.41**
AChE	99.75 ± 2.80	71.65 ± 2.08**	101.15 ± 2.09	66.70 ± 2.81**
ChAT	100.01 ± 9.82	30.91 ± 4.25**	100.01 ± 7.34	35.00 ± 7.30**
Serotonergic system				
5-HT	52.95 ± 3.25	23.95 ± 1.39**	58.95 ± 4.09	28.26 ± 1.25**
5-HIAA	201.10 ± 19.91	94.59 ± 8.18**	237.20 ± 20.02	94.70 ± 7.81**

Values are mean ± S.E.M. ACh (acetylcholine) and choline are expressed in nmol/mg of wet tissue, AChE (acetylcholinesterase) and ChAT (cholinacetyltransferase) are expressed as percentaje of control values and, 5-HT (serotonin) and 5-HIAA (5-hydroxyindolacetic acid) are expressed in pg/mg of wet tissue. BA10 (Brodmann area 10) and BA20 (Brodmann area 20) from control ($n = 19$–20) and Alzheimer's disease patients ($n = 20$–22).

** Significantly lower than control, Student's *t*-test, $P < 0.01$.

3.2. Relationship between neurochemical variables with the cognitive status

In AD patients mean MMSE score, and the MMSE decline before death were 5 ± 1 and 10 ± 2, respectively. Spearman's rank correlation showed a statistically significant positive correlation between final MMSE score and ChAT activity both in BA10 ($n = 20$; $r = 0.453^*$; $P = 0.045$) and BA20 ($n = 19$; $r = 0.639^{**}$; $P = 0.003$) in AD patients. AChE activity was also correlated with final MMSE scores in the frontal ($n = 20$; $r = 0.573^{**}$; $P = 0.008$) as well as in temporal ($n = 21$; $r = 0.532^*$; $P = 0.013$) cortex.

The ChAT/5-HT ratio showed significant correlations with MMSE scores both in BA10 and BA20 (Fig. 1), and similar results were found even though MMSE scores of 0 were removed. When samples were separated by gender of the patients, the ratio between AChE and 5-HT levels in BA20 was also correlated to MMSE decline in case of women ($n = 12$; $r = 0.534^*$; $P = 0.044$). Moreover, women also showed a significant correlation between ACh/5-HT and MMSE decline ($n = 11$; $r = 0.720^*$; $P = 0.019$) in BA20.

3.3. Relationship between neurochemical variables with behavioral syndromes

Final scores for BPSD assessed by PBE in AD patients were as follows: depression factor 3 ± 0.3; overactivity 3 ± 0.4; psychosis 2 ± 0.3; aggressive behavior 5 ± 0.4.

Stepwise multiple regression indicated that the best predictor of lowered ChAT and AChE levels both in BA10 and BA20 was aggressive behavior scores among all four factors studied (Table 3). Aggressive behavior score and MMSE score showed a statistically significant negative correlation in AD patients ($n = 20$, $r = -0.707^{**}$; $P < 0.001$).

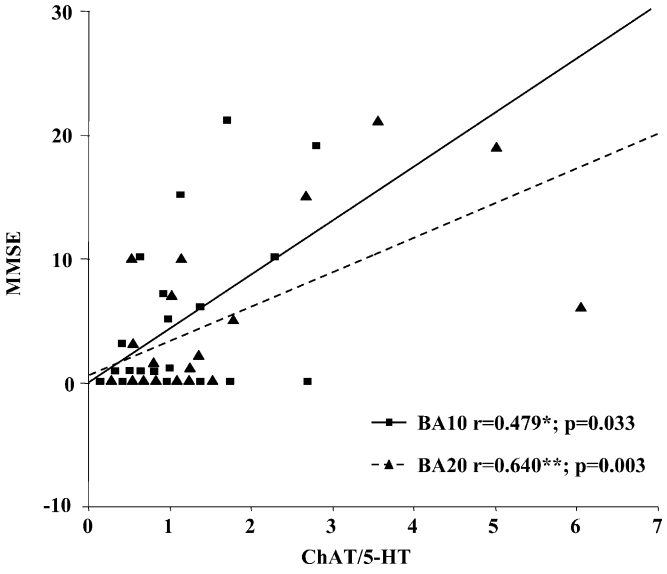

Fig. 1. Correlations between ChAT/5-HT ratio and MMSE from AD (Alzheimer's disease) in BA10 (Brodmann area 10) and BA20 (Brodmann area 20) (n = 19–20). ChAT (cholinacetyltransferase), 5-HT (serotonin). * or ** Significant Spearman's rank correlations ($P < 0.05$ or $P < 0.01$).

Table 3
Step-wise multiple regression between neurochemical determinations and behavioral and psychological syndromes in dementia

	Aggression	Overactivity	Psychosis
AChE (BA10)			
Adj r^2	0.275*		
P	0.018		
AChE (BA20)			
Adj r^2	0.251*		
P	0.023		
ChAT (BA10)			
Adj r^2	0.231*		
P	0.029		
ChAT (BA20)			
Adj r^2	0.321*		
P	0.012		
5-HT (BA10)			
Adj r^2		0.220*	
P		0.038	
5-HT (BA20)			
Adj r^2			0.221* (0.389*)
P			0.028 (0.024)
AChE/5-HT (BA20)			
Adj r^2			0.236* (0.463*)
P			0.026 (0.013)

Adj r^2 (adjusted r^2), P (statistical signification), AChE (acetylcholinesterase), ChAT (cholineacetyltransferase), 5-HT (serotonin), BA10 (Brodmann area 10), BA20 (Brodmann area 20) ($n = 20$). Parenthesis data corespond to women values ($n = 10$). Depression was not included as no significant step-wise multiple regression was found between neurochemical data and this behavioral factor.

* Significant stepwise multiple regression.

The best predictor for 5-HT reductions in BA10 was overactivity factor (Table 3). On the other hand, in BA20, 5-HT levels and the ratio AChE to 5-HT levels were best predictors for the psychotic factor. When separated by gender, these correlations were due to women (Table 3).

4. Discussion

Reflecting the loss of cholinergic innervation, reductions in AChE and ChAT have been reported in AD brains (Francis et al., 1999; Giacobini, 2003). As expected, profound depletions in all cholinergic markers measured (ACh, choline, ChAT, AChE) were found both in frontal and temporal cortex from AD patients in the present study. Important limitations to consider in the study are the *post-mortem* delay and available clinical details. However, similar *post-mortem* delays to those used in the present study have been described in the literature to measure biochemical markers (i.e. Lai et al., 2001). Due to the labile nature of ACh in *post-mortem* tissue, data on ACh levels have been taken cautiously.

Cholinergic degeneration in AD has been widely associated with cognitive impairments in the illness (Terry &

Buccafusco, 2003). In keeping with this argument, in our study, ChAT and AChE levels were correlated to final MMSE scores in AD. Supporting these data, AChE inhibitors such as rivastigmine and donezepil have been shown to maintain MMSE scores for up to 52 weeks in placebo-controlled study (Tariot, 2001).

Regarding to the serotonergic system, and as previously reported, a marked depletion in 5-HT and 5-HIAA levels both in frontal and temporal cortex from AD patients was observed. As well as pathology within synapses, considerable loss of 5-HT in the cortex is likely due to loss of projections from the raphe, as it has long been recognised that in AD these nuclei are subject to neurofibrillary tangle formation. We found no correlation between concentrations of 5-HT and cognitive status. This was a somehow unexpected result as, in keeping with the implication of the serotonergic system in cognition, it has been described that 5-HT levels correlated negatively with the rate of MMSE decline in the frontal cortex (Lai et al., 1996). However, it has also been described that not all patients with AD had depleted raphe neurons, indicating that raphe neuron loss is not essential for cognitive decline (Halliday et al., 1992). It could be argued that plasticity of the serotonergic system enables surviving raphe neurons to successfully take over the function of those cells that are lost until an imbalance between the serotonergic–cholinergic systems leads to cognitive impairments (Chen et al., 2000). In keeping with this idea, we have analyzed the ratio between cholinergic/serotonergic levels, e.g. a maintained ratio in a degenerating cholinergic system (as in AD) would be achieved through a decrease in the serotonergic input. We found that the quotient between ChAT and 5-HT both in frontal and temporal cortex was correlated to final MMSE scores. Moreover the ratios AChE/5-HT and ACh/5-HT were also correlated with MMSE decline at least in women. Taking into account that 5-HT seems to act as an inhibitory neurotransmitter on cholinergic neurons, decreases in the serotonergic tone would help to maintain the cholinergic input in cholinoceptive deficient target areas (Garcia-Alloza et al., 2004). However, it is to note that a ratio between a pair of neurotransmitters may not be the best approach, as the ratio may not change as they both decline, and therefore, these data should be considered with caution.

Imperfect integration of memory and cognitive function may contribute to BPSD that most patients suffer in AD (Parnetti et al., 2001). Increasing evidence suggests that cholinergic deficiency in cortical regions might be linked to BPSD observed in AD patients (Minger et al., 2000). This contention is supported by the fact that anticholinergic drugs exacerbate many of the behavioral abnormalities associated to AD, and treatment with cholinergic compounds often improves behavior. These observations provide the basis for the cholinergic hypothesis of the neuropsychiatric symptoms of AD (Cummings, 2000; Levy et al., 1999). In our study, in keeping with findings from Minger et al. (2000), the best predictor of low ChAT and AChE levels was agressive behavior. As ChAT and AChE decreases in AD also correlate to cognitive impairment it could be argued that the relationship between cholinergic deficits and aggressive behavior may be because of a potential relationship between the behavioral syndrome and cognitive impairment. Supporting this idea, we have found a significant correlation between the final MMSE score and aggressive behavior.

We found that the best predictor for 5-HT levels in frontal cortex (BA10) was overactivity factor. On the other hand the best predictor for 5-HT levels in temporal cortex (BA20) was psychotic factor. Previous studies showed that psychosis was associated with reductions in 5-HT in different brain regions (Zubenko et al., 1991) and delusions and hallucinations were observed in patients with lower cell counts in the dorsal raphe nucleus (Forstl, Burns, Levy, & Cairns, 1994). It is unclear why overactivity factor is correlated to 5-HT levels in BA10 while correlations with the psychotic factor appear in BA20, although possible explanations may include functional specialization of the frontal and temporal cortex, just like it has been already suggested for other BPSD in AD patients (Mitchell, Colledge, Leonard, & Blair, 2002). Psychotic manifestations in AD have been associated with pathology in the temporal lobe (Forstl et al., 1994; Zubenko et al., 1991) and there is also evidence that implicates temporal areas in the production of hallucinations (Woodruff, Wright, & Bullmore, 1997).

In the temporal cortex (BA20) of women, the ratio 5-HT/AChE was also correlated to the psychotic factor and therefore, women with AD may have a higher incidence of delusions. It could be suggested that the profile of delusions and hallucinations seen is different from that seen in schizophrenia and the reason for these differences in AD may be linked to the cholinergic deficit. Indeed, the presence of psychotic symptoms in AD has been shown to be a predictor of faster cognitive decline (Stern et al., 1994) and it has been described the effectiveness of AChE inhibitors in treating delusions or hallucinations (Morris et al., 1998).

The hypothesis of this study was that imbalance between the cholinergic and serotonergic systems is related to cognitive symptoms and BPSD in patients with AD. It seems increasingly clear that the neuropsychiatric symptoms seen in AD are not simply a consequence of neurodegeneration, but probably result from different or multiple neurotransmitter pathologic features, of which some patients are more at risk than others. The major finding of this study is of relationships between neurochemical markers of both cholinergic and 5-HT systems and non-cognitive behavioral disturbance in patients with dementia. In this work we have shown that cortical cholinergic AChE could be considered not only a putative marker of accelerated cognitive decline in AD at the end of life, but it may be also implicated in BPSD such as aggressive behavior. Our results seem to support the use of cholinesterase inhibitors in alleviating non-cognitive behavioral symptoms in patients with AD. On the other hand 5-HT levels in temporal cortex seem to be a good predictor of psychotic factor associated to AD, although this implication might be mediated by cholinergic disruption. Traditional treatments for

psychosis in AD are neuroleptics, strong dopamine D2 antagonists, associated with extrapyramidal symptoms and that may have anticholinergic side effects. Although atypical antipsychotic drugs, such as risperidone (Defilippi & Crismon, 2000) represent alternative drugs for treating BPSD, it could also be suggested that in pharmacotherapy of AD, attempts to restore deficits of the transmitter systems might be directed to the serotonergic system.

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