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Morphological and Biochemical Changes in the Cholinergic and Monoaminergic Systems in Alzheimer-type Dementia

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Summary. The integrity of cholinergic and monoaminergic neuronal systems in Alzheimer-type dementia (ATD) was studied using a combination of morphological and biochemical procedures applied to samples from seven ATD brains and ten control brains. On morphological examination, the number of neurons was counted in the nucleus basalis of Meynert (nbM), the locus coeruleus (LC) and the nucleus centralis superior (NCS). Biochemically, the activities of choline acetyltransferase (ChAT) and the concentrations of noradrenaline (NA) and serotonin (5-hydroxytryptamine; 5-HT) were measured in the nbM, LC, NCS, frontal cortex, temporal cortex and occipital cortex. Compared with the controls, the mean number of neurons in the nbM and LC were significantly reduced in the ATD brains. The neuronal loss in the NCS in the ATD brains was not significant. The ChAT activities in all regions from the ATD brains had a tendency to be reduced. Marked reductions in the NA concentrations in the LC and 5-HT concentrations in the temporal and occipital cortices were found in the ATD brains. These findings suggest that various neurotransmitter systems, including cholinergic, noradrenergic and serotonergic systems, are affected in the ATD brains.

Key words: Alzheimer-type dementia – Cytometry – Cholinergic system – Monoaminergic system

Introduction

A number of biochemical studies of postmortem brains from patients with Alzheimer-type dementia (ATD) have been performed in an attempt to unveil the pathogenesis of this disorder. The reduction of various neurotransmitters and their associated enzyme markers has been demonstrated (Arai 1985).

Neuropathologically, the degeneration and loss of the neurons in the nucleus basalis of Meynert (nbM) (Whitehouse et al. 1982; Arendt et al. 1983; Tagliavini and Pilleri 1983; presumed to be cholinergic neurons), in the locus coeruleus (LC) (Tomlinson et al. 1981; Bondareff et al. 1982; Iversen et al. 1983; Mann et al. 1984; noradrenergic neurons), and in the raphe nuclei (Curcio and Kemper 1984; Yamamoto and Hirano 1985; serotonergic neurons) have been observed.

Although all these various neurotransmitter systems may be damaged in the ATD brains, there has so far been no report in which these systems have been studied both morphologically and biochemically in the same patients. In this investigation, we have studied the integrity of the cholinergic, noradrenergic and serotonergic systems in the ATD brains by combining morphological analysis and biochemical assay techniques.

Materials and Methods

Human brains were obtained at autopsy from seven ATD patients, in whom the diagnosis of ATD was neuropathologically confirmed, and ten age-matched subjects without any history of neurological or psychiatric illness (five of the control subjects were available for the morphological examination). Clinical and postmortem data for both groups are presented in Table 1.

The brains were removed at autopsy and divided midsagitally. In each brain, one himisphere was fixed in 10% formalin for neuropathological examinations, while the other was rapidly frozen and stored at −70°C for subsequent biochemical examinations.

Neuropathological Examinations

The total number of neurons in the nbM, LC and raphe nuclei (RA) was counted. The following sections were chosen for neuronal counts: those at the levels of the broadest portion of the nbM, of the central part of the LC, and of the nucleus centralis superior (NCS) (Olszewski and Baxter 1982). To

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Table 1. Clinical and postmortem data

	Alzheimer-type dementia	Control
Number of brains	7	10
Sex (male:female)	3:4	8:2
Age at death (years)	67.2 ± 16.5[a]	70.0 + 12.0
Postmortem delay (h)	8.1 + 4.9	8.4 ± 6.2

[a] Values are mean ± SD

compare these data with our previous biochemical data (Arai et al. 1984a), we selected the NCS to represent the RA.

In the nbM, only large neurons (at least 20 μm in diameter) which had nucleolei and prominent Nissl substance were counted. In the LC, all pigmented neurons with nucleolei were counted. In the NCS, both medium- and small-sized neurons were counted, however only neurons with nucleolei and Nissl substance were included. Neuronal counts in these nuclei were performed on the Nissl preparations.

The number of dopaminergic neurons in the substantia nigra was not counted here, since this nucleus was pathologically intact in all ATD cases.

Biochemical Examinations

The activities of choline acetyltransferase (ChAT), as well as the concentrations of noradrenaline (NA) and serotonin (5-hydroxytryptamine; 5-HT), were measured in the nbM, LC, NCS, frontal (Brodmann area 10), temporal (Brodmann area 20) and occipital cortices (Brodmann area 17). Full details of the methods have been given previously (Arai et al. 1984a, b).

Statistical Analysis

Student's *t*-test and Pearson's correlation technique were used for statistical analysis.

Results

The mean number of neurons in the nbM and the LC in the ATD brains was significantly lower than in the control brains ($p<0.01$; Table 2, Fig. 1). However, there was no difference in the mean number of neurons in the NCS between the ATD and the controls (Table 2).

The mean ChAT activities in all regions of ATD brains tended to be reduced. The mean concentrations of NA of the ATD brains were significantly lower in the LC ($p<0.05$) than those of the controls (Table 3). The mean concentrations of 5-HT in the temporal and occipital cortices from the ATD brains were reduced significantly compared with those from the control brains ($p<0.05$ in the temporal and occipital cortices; Table 3).

There were significant correlations between the number of neurons in the nbM and ChAT activities in the nbM ($r=0.85$, $n=10$, $p<0.05$), in the frontal cortex ($r=0.74$, $n=10$, $p<0.05$), and in the occipital cortex ($r=0.81$, $n=10$, $p<0.01$). There were also significant correlations between the number of neurons in the LC and NA concentrations in the LC ($r=0.93$, $n=9$, $p<0.001$) and in the frontal cortex ($r=0.83$, $n=8$, $p<0.01$). However, no correlation was found between the number of neurons in the NCS and 5-HT concentrations in any region.

Table 2. Number of neurons in the nucleus basalis of Meynert (nbM), locus coeruleus (LC) and nucleus centralis superior (NCS)

Case	Age (years)	Duration (years)	Number of neurons		
			nbM	LC	NCS
1	45	5	159	6	115
2	48	8	88	11	–
3	61	7.5	139	3	61
4	70	17	142	27	122
5	76	18	241	76	–
6	84	7	188	10	138
7	86.5	2	298	47	124
Mean ($n=7$)	67.2 ± 16.5	9.2 ± 6.0	189.0* ± 76.3	25.7* ± 26.9	112.0 ± 29.7
Mean of controls ($n=5$)[a]	65.2 ± 11.8	0	427.8 ± 127.4	83.0 ± 8.7	141.8 ± 20.7

Cases 1–4 belong to presenile onset group and cases 5–7 to senile onset group

* $P<0.01$

[a] Five subjects out of the controls were available for the morphological examination

Discussion

Our observations of the loss of nbM neurons and the reduction of ChAT activities are in good agreement with the earlier observations (Bowen et al. 1976; Davies and Maloney 1976; Perry et al. 1977; Whitehouse et al. 1982).

Similarly, our data also confirm reports of noradrenergic neuron loss from the LC in ATD (Tomlinson et al. 1981; Bondareff et al. 1982; Iversen et al. 1983; Mann et al. 1984). It is also interesting to note that our finding of the most marked loss of LC neurons is observed in presenile patients agrees with the results of Bondareff et al. (1982).

The serotonergic system has, however, been less extensively studied in ATD, although there have been a few reports of the extensive loss of the large neurons in nucleus raphe dorsalis (NRD) from ATD (Curcio and Kemper 1984; Yamamoto and Hirano 1985). In this study, we also observed damage to the raphe nuclei (accumulation of neurofibrillary tangles) and a loss of cortical 5-HT content, but in the raphe area (NCS) we did not observe any depletion of neuronal numbers. This possibly reflects the lower density of

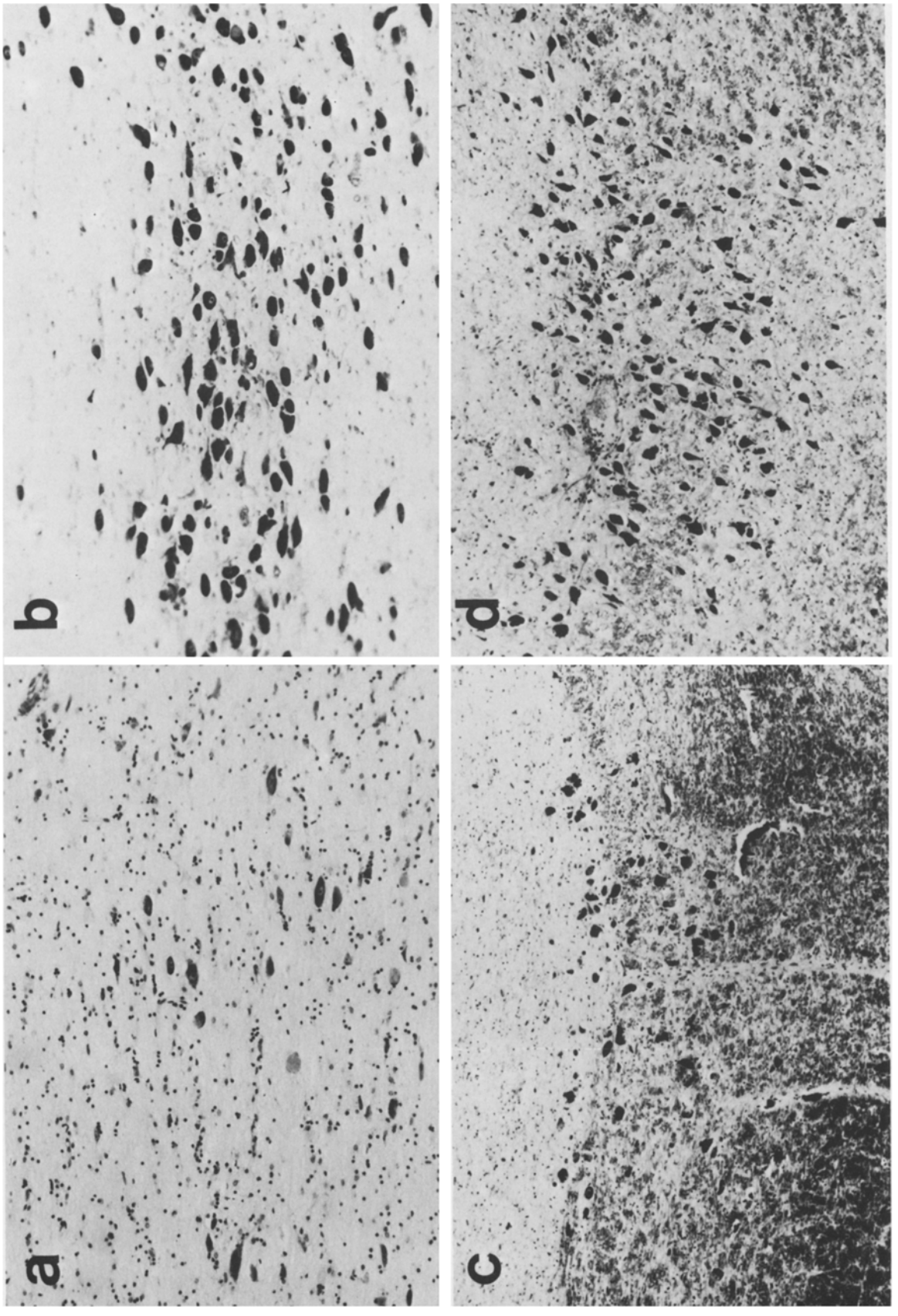

Fig. 1 a–d. Neuropathological findings in the nucleus basalis of Meynert (nbM) and locus coeruleus (LC). **a** Marked neuronal loss in the nbM of an Alzheimer-type dementia (ATD) case. **b** Neurons in the nbM of a control case. **c** Apparent neuronal loss in the LC of an ATD case. **d** Neurons in the LC of a control case. Nissl stain, **a, b** ×93; **c, d** ×37

Table 3. Mean values of choline acetyltransferase (ChAT) activities, and noradrenaline (NA) and serotonin (5-HT) concentrations

	ChAT				NA			5-HT			
	nbM	F	T	O	LC	F	O	NCS	F	T	O
ATD	4.45 ± 4.47 (7)	0.94 ±1.27 (7)	0.74 ±0.94 (6)	0.63 ±0.76 (7)	4.12* ±4.08 (6)	0.06 ±0.08 (4)	0.12 ±0.11 (4)	8.23 ±6.82 (7)	0.04 ±0.05 (6)	0.03* ±0.03 (5)	0.13* ±0.12 (7)
Control	11.63 ±12.60 (9)	1.38 ±1.24 (10)	1.02 ±0.86 (10)	1.34 ±1.30 (10)	10.51 ±4.82 (8)	0.13 ±0.11 (9)	0.16 ±0.11 (9)	13.17 ±10.75 (9)	0.13 ±0.09 (7)	0.13 ±0.13 (7)	0.40 ±0.29 (10)

All these data have been evaluated in the same patients. Activities are expressed as n mol/h per mg protein, and concentrations are expressed as ng/mg protein. The numbers in the brackets are numbers of cases. F, T and O: frontal, temporal and occipital cortices respectively

* $P < 0.05$

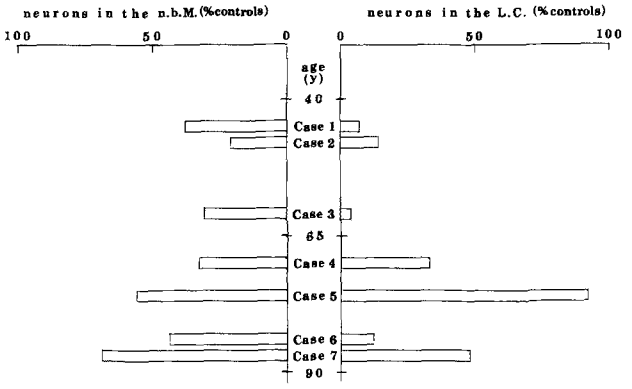

Fig. 2. Number of neurons in the nbM and LC of ATD. All values are expressed as percentage of the mean of controls for each regions

5-HT neurons in this area (NCS) than in NRD. More extensive studies in the raphe nuclei of ATD brains should be made to demonstrate the damage to this system more clearly.

Our data, therefore, show clearly that, in the ATD cases examined here, there is damage to all the described main ascending inputs to the associate cortex (cholinergic, noradrenergic and serotonergic neurons). Following the original observation of the cholinergic deficit in ATD (Bowen et al. 1976; Davies and Maloney 1976; Perry et al. 1977), it has been suggested that specific and selective damage of only the cholinergic system might underlie the pathology of ATD (so-called "cholinergic hypothesis"). However, our results do not support this hypothesis, for they disclose that, in addition to the damage to the cholinergic system, the monoaminergic systems were also affected in the ATD brains.

Concerning the damage to the noradrenergic system in ATD, Perry et al. (1981) have speculated that the damage to that system might be a later (secondary) change. In our study, however, the noradrenergic system was more severely affected than the cholinergic system in five patients out of the ATD group examined, when the severity of neuronal loss was compared between LC and nbM in each brain (Fig. 2). Mann et al. (1984) also reported similar findings in the younger group of the ATD patients. Therefore, these results suggest that the damage to the noradrenergic system is not a secondary change to the cholinergic deficit. It is also interesting that the three ATD patients with presenile onset had more severe damage to the noradrenergic neurons than the senile-onset ATD patients (Fig. 2), although there was no significant difference in duration of illness between the presenile and senile groups. It has been reported that younger ATD patients had more severe damage to the cholinergic (Davies 1979), noradrenergic (Bondareff et al. 1982) and serotonergic neurons (Arai 1985). These findings support the traditional neuropsychiatric nosology that ATD patients are divided into two subgroups: the presenile and senile-onset groups.

In conclusion, the present morphological and biochemical findings show clearly for the first time that, in the same patients, cholinergic, noradrenergic and serotonergic systems are damaged in ATD.

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