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# Monoamine Neurotransmitters and Their Metabolites in Brain Regions in Alzheimer's Disease: A Postmortem Study

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**KEY WORDS:** Alzheimer's disease; postmortem brain; neurotransmitters; catecholamines; indoleamines; acid metabolites.

## SUMMARY

1. Concentrations of the neurotransmitter amines noradrenaline (NA), dopamine (DA), and 5-hydroxytryptamine (5-HT) and the acid metabolites homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were determined in four regions of postmortem brains of demented patients with or without Alzheimer's disease (AD).

2. NA was deficient in the temporal cortex (BA 21) of AD, but not of non-AD, patients.

3. Caudate, in particular, had an impaired dopaminergic system in AD patients, with low HVA levels.

4. In all regions investigated [amygdala, caudate, putamen, temporal cortex (BA 21)] 5-HT was significantly depleted in AD patients, and 5-HIAA was also depleted in amygdala and caudate.

5. These results indicate that neurotransmitter systems other than cholinergic systems are also widely affected in AD and suggest that these deficits may also play an important role in determining the symptomatology of AD.

## INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia (Tomlinson and Corsellis, 1984). It is characterized by the presence of neurofibrillary tangles,

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neuritic plaques, and argyrophilic inclusion bodies. Also notable is the gross atrophy of the cerebral cortex (DeJong and Pope, 1975; Rossor, 1982). This neuronal degeneration in AD has a profound effect on brain chemistry, resulting in a marked decrease in activity of choline acetyltransferase in the amygdala, caudate nucleus, cerebral cortex, and hippocampus (Davies and Maloney, 1976; Perry *et al.*, 1977; Spillane *et al.*, 1977; Whitehouse *et al.*, 1981). However, other neurotransmitter systems apart from the cholinergic system are also known to be affected in AD (Hardy *et al.*, 1985; Quirion *et al.*, 1986; Perry, 1987; Rossor and Iversen, 1986; Baker and Reynolds, 1989; Gottfries, 1990; Selkoe, 1991). Neuropathological and biochemical studies on human postmortem brains have revealed a loss of noradrenergic neurons in the locus coeruleus (Bondareff *et al.*, 1981; Iversen *et al.*, 1983) with the resultant depletion of noradrenaline (NA) in the cortex (Adolfsson *et al.*, 1979; Arai *et al.*, 1984; Iversen *et al.*, 1983; Mann *et al.*, 1982; Winblad *et al.*, 1982). A significant loss of the neuropeptide somatostatin has been reported, with the greatest deficit occurring in the temporal cortex (Davies *et al.*, 1980; Ferrier *et al.*, 1983; Rossor *et al.*, 1980). Depleted concentrations of the indoleamine 5-hydroxytryptamine (5-HT) and its major metabolite 5-hydroxindoleacetic acid (5-HIAA) (Adolfsson *et al.*, 1979; Arai *et al.*, 1984; Bowen *et al.*, 1983; Winblad *et al.*, 1982) together with a reduction in 5-HT receptors (Bowen *et al.*, 1983; Reynolds *et al.*, 1984) have also been reported in the cerebral cortex of AD subjects.

We have been investigating concentrations of biogenic amine neurotransmitters and their metabolites in regions of postmortem brains from AD patients and have previously reported (Baker and Reynolds, 1989) that levels of NA, 5-HT, and 5-HIAA are depleted in the hippocampus but not the substantia innominata of these patients. These investigations have now been extended to other brain areas (amygdala, caudate nucleus, putamen, and temporal cortex Brodmann area 21) and postmortem tissue from non-AD demented subjects (i.e., dementia without the neuropathology of AD) included. The results of those investigations are reported here.

## MATERIALS AND METHODS

Diagnoses of AD and non-AD cases were based on neuropathological examinations of brain tissue. In all cases the Alzheimer's patients clearly had a significant number of plaques and neurofibrillary tangles compared to both control subjects and demented patients with non-AD disease. Subsequent neurochemical analysis of the affected brain regions correlated with neuropathological findings (Tables I–IV). The ages of demented patients with AD and non-AD ranged from 66 to 93 years ($81 \pm 8.2$ years, mean $\pm$ S.D.) and 63 to 96 years ($81.8 \pm 9.4$), respectively. The ages of control subjects ranged from 51 to 91 years ($72.7 \pm 13.1$ years). The ratios of male-to-female subjects in AD and non-AD patients with dementia were 1:12 and 1:11, respectively, and the ratio of male-to-female subjects in the control group was 5:5. The postmortem delay

**Table I.** Concentrations of Neurotransmitter Amines and Metabolites in the Amygdala from Control Subjects and Demented Patients[a]

| | Controls | Demented patients | |
|---|---|---|---|
| | | AD | Non-AD |
| Noradrenaline | 80.7 ± 13.8(9) | 50.6 ± 17.8(13) | 69.4 ± 15.4(12) |
| Dopamine | 54.7 ± 20.1(9) | 58.9 ± 12.3(13) | 95.2 ± 35.5(12) |
| Homovanillic acid | 765 ± 110(9) | 672 ± 84.9(13) | 694 ± 90.2(12) |
| 5-Hydroxytryptamine | 176 ± 22.5(9) | 90.3 ± 12.5(13)* | 176 ± 29.1(12)** |
| 5-Hydroxyindoleacetic acid | 589 ± 76.2(9) | 336 ± 42.4(11)* | 508 ± 66.5(12) |

[a] Results are expressed as mean ± SE ng/g tissue, and the sample number ($N$) is shown in parentheses. AD, Alzheimer's disease; non-AD, non-Alzheimer's disease.
* $P < 0.05$ compared to control subjects.
** $P < 0.05$ compared to AD subjects.

**Table II.** Concentrations of Neurotransmitter Amines and Metabolites in the Caudate Nucleus from Control Subjects and Demented Patients[a]

| | Controls | Demented patients | |
|---|---|---|---|
| | | AD | Non-AD |
| Dopamine | 4178 ± 972(10) | 2868 ± 720(13) | 4079 ± 915(12) |
| Homovanillic acid | 6981 ± 908(10) | 3744 ± 464(13)* | 5450 ± 912(12) |
| 5-Hydroxytryptamine | 395 ± 57(10) | 177 ± 38.3(13)* | 274 ± 47.7(12) |
| 5-Hydroxyindoleacetic acid | 1036 ± 181.8(10) | 529 ± 95.5(13)* | 716 ± 89.1(12) |

[a] Results are expressed as mean ± SE ng/g tissue, and the sample number ($N$) is shown in parentheses. AD, Alzheimer's disease; non-AD, non-Alzheimer's disease.
* $P < 0.05$ compared to control subjects.

**Table III.** Concentrations of Neurotransmitter Amines and Metabolites in the Putamen from Control Subjects and Demented Patients[a]

| | Controls | Demented patients | |
|---|---|---|---|
| | | AD | Non-AD |
| Dopamine | 6,073 ± 667(10) | 6,278 ± 1,135(13) | 7,260 ± 1,269(11) |
| Homovanillic acid | 11,471 ± 1,619(10) | 10,802 ± 1,385(13) | 12,082 ± 1,209(11) |
| 5-Hydroxtryptamine | 511 ± 55.8(10) | 326 ± 44.9(13)* | 472 ± 40.4(11)** |
| 5-Hydroxyindoleacetic acid | 1,715 ± 218(10) | 1,391 ± 186(13) | 1,569 ± 167(11) |

[a] Results are expressed as mean ± SE ng/g tissue, and the sample number ($N$) is shown in parentheses. AD, Alzheimer's disease; non-AD, non-Alzheimer's disease.
* $P < 0.05$ compared to control subjects.
** $P < 0.05$ compared to AD subjects.

times for AD and non-AD dementia subjects were 29.9 ± 20.8 and 34.5 ± 26 hr, respectively, and those of control subjects were 33.2 ± 21.3 hr. The neuropathology of non-AD subjects with dementia varied, with one subject having senile cerebral atrophy with arteriosclerosis and hippocampal damage from hypoxic/hypotensive episode, another subject with encephalitis, and yet another with ateriosclerosis. A single subject had a multiinfarct dementia, and in the remaining subjects the cause of dementia was unknown. Control tissue was taken

**Table IV.** Concentrations of the Neurotransmitter Amines and Metabolites in the Temporal Cortex (BA 21) from Control Subjects and Demented Patients[a]

| | | Demented patients | |
|---|---|---|---|
| | Controls | AD | Non-AD |
| Noradrenaline | 8.3 ± 1.5(10) | 2.4 ± 0.4(12)* | 6.9 ± 1.5(11)** |
| Dopamine | 2.2 ± 0.5(10) | 2.8 ± 0.5(13) | 3.3 ± 0.9(11) |
| Homovanillic acid | 162 ± 33.3(10) | 143 ± 17.6(13) | 129 ± 11.1(12) |
| 5-Hydroxytryptamine | 11.3 ± 1.9(10) | 4.2 ± 1.2(13)* | 9.3 ± 1.4(12)** |
| 5-Hydroxyindoleacetic acid | 108 ± 19.4(10) | 67.5 ± 13.9(13) | 89.3 ± 11.9(12) |

[a] Results are expressed as mean ± SE ng/g tissue, and the sample number ($N$) is shown in parentheses. AD, Alzheimer's disease; non-AD, non-Alzheimer's disease.
* $P < 0.05$ compared to control subjects.
** $P < 0.05$ compared to AD subjects.

from subjects with no history of neurological or psychiatric disease or of psychoactive drug treatment.

Dissection and collection of tissue samples was performed essentially as described by Spokes (1979). In brief, brains were sagitally dissected, with one hemisphere retained for histological examinations. The remaining second half of the brain was placed in a −20°C freezer for a period not longer than 96 hr, and thereafter prolonged storage was at −70°C. Prior to dissection, frozen brains were transferred to a −20°C freezer for a period of 12 hr and tissue sections cut with an electric meat slicer under a biohazard flow hood. Coronal sections of the brain (5-mm-thick slices) were taken beginning from the frontal pole and the sections placed on a refrigerated surface at −10°C. After locating anatomical landmarks, identified brain areas were dissected and chopped into fine pieces, mixed, and stored in plastic tubes at −70°C. Brain regions were homogenized in ice-cold perchloric acid (0.1 $M$) containing 0.2 m$M$ EDTA and 0.1 m$M$ ascorbic acid at a tissue concentration of 100 mg/ml. Centrifugation (12,000 rpm) was performed for 3 min at room temperature to precipitate the protein. The supernatants were analyzed for neurotransmitter and metabolite concentrations with a high-performance liquid chromatograph (HPLC) equipped with a Spherisorb 5 ODS column (length, 25 cm; internal diameter, 4.6 mm) at 40°C and attached to an electrochemical detector (Reynolds, 1983).

## RESULTS AND DISCUSSION

Data are presented in Tables I–IV. These data were analyzed using one-way analysis of variance, followed by Newman–Keuls multiple-comparisons tests where appropriate. The critical two-tailed probability for significance was $P < 0.05$. Results indicate that 5-HT [$F(2,31) = 5.35$, $P < 0.05$] and 5-HIAA [$F(2,29) = 4.15$, $P < 0.05$] are depleted in the amygdala in AD compared to control subjects (Table I). Concentrations of NA in amygdala were also depleted but these did not reach significance. None of the amines or metabolites measured was depleted in the non-AD patients relative to controls.

Brain concentrations of neurotransmitters and metabolites in the caudate nucleus and putamen are illustrated in Tables II and III. The DA metabolite HVA [$F(2,32) = 4.46$, $P < 0.05$] and the neurotransmitter 5-HT [$F(2,32) = 5.29$, $P < 0.05$] and its metabolite 5-HIAA [$F(2,32) = 4.35$, $P < 0.05$] were all significantly depleted in caudates of AD subjects compared to the control cases. DA levels were lower in caudates of AD subjects but these did not reach significance. Interestingly, only 5-HT [$F(2,31) = 4.56$, $P < 0.05$] was deficient in the putamen of AD subjects. None of these depletions was evident in the non-AD cases. In Brodmann area 21 (Table IV), NA [$F(2,30) = 6.85$, $P < 0.05$] and 5-HT [$F(2,32) = 6.33$, $P < 0.05$] were both significantly lower in AD subjects only. Results therefore suggest a different etiology of dementia in AD compared to that in non-AD subjects. With AD subjects it seems likely that NA, 5-HT, and 5-HIAA play an important role in determining the symptoms of this disease. The loss of NA in AD patients is in agreement with previous reports (Mann *et al.*, 1980; Gottfries *et al.*, 1983; Bondareff *et al.*, 1981; Arai *et al.*, 1984). Depleted levels of the NA metabolite 3-methoxy-4-hydroxyphenlethylglycol (MHPG) (Cross *et al.*, 1983), reduced DA $\beta$-hydroxylase activity (Cross *et al.*, 1981), and degeneration of the locus coeruleus (Tomlinson *et al.*, 1981) have all been reported in AD cases. The dopaminergic system also appears to be altered, particularly in the caudate nucleus of AD subjects. Low levels of HVA are evident in the caudates of AD subjects. This is in agreement with the report of Gottfries *et al.* (1983). Reduced HVA levels have also been reported to occur in the CSF of AD patients (Gottfries, 1979). It has been reported that up to 50% of AD patients exhibit Parkinsonian symptoms (Pearce, 1974), and conversely between 11 and 53% of Parkinson patients exhibit dementia (Gottfries *et al.*, 1980). Therefore, as pointed out by Gottfries *et al.* (1980), an abnormal DA metabolism could be the pathogenic cause for motor deficits in AD subjects. Another interesting aspect demonstrated by Gottfries (1981) is that a negative correlation exists between HVA concentration in the caudate and intellectual impairment, which raises important questions about possible dopaminergic involvement in intellectual functions.

The neurotransmitter 5-HT was depleted in all four regions investigated in the AD brains. These results are in agreement with a number of other reports (Adolfsson *et al.*, 1979; Winblad *et al.*, 1982 Bowen *et al.*, 1983; Gottfries *et al.*, 1983; Perry, 1987; Arai *et al.*, 1984), although in a previous report from our laboratories we did not find a depletion of 5-HT in the substantia innominata (Baker and Reynolds, 1989). It is likely that the serotonergic system is more widely affected than the catecholaminergic systems, as all regions investigated in the present study show a deficit of 5-HT. This could be due to a widespread degeneration of the serotonergic neurons in AD subjects. In this respect it is interesting to note that the neurons of the raphé nuclei are frequently known to have neurofibrillary tangles in AD cases (Ishii, 1966).

In conclusion, it can be said from these data that both the catecholaminergic and the serotonergic systems are extensively affected in AD. Hence the consequence of neurodegeneration in AD cannot merely be explained by the loss of the cholinergic neurons, and a more general interpretation has to be

considered, where a complex interaction between the various neurotransmitter systems is more likely to be responsible for the wider symptomatology commonly seen in AD.

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## REFERENCES

Adolfsson, R., Gottfries, C. G., Roos, B. E., and Winblad, B. (1979). Changes in the brain catecholamines in patients with dementia of Alzheimer type. *Br. J. Psychiat.* **135**:216–223.

Arai, H., Kosaka, K., and Iizuka, R. (1984). Changes of biogenic amines and their metabolites in postmortem brains from patients with Alzheimer-type dementia. *J. Neurochem.* **43**:388–393.

Baker, G. B., and Reynolds, G. P. (1989). Biogenic amines and their metabolites in Alzheimer's disease: Noradrenaline, 5-hydroxytryptamine and 5-hydroxyindole-3-acetic acid depleted in hippocampus but not in substantia innominata. *Neurosci. Lett.* **100**:335–339.

Bondareff, W., Mountjoy, C. Q., and Roth, M. (1981). Selective loss of neurons of origin of adrenergic projection to cerebral cortex (nucleus locus coeruleus) in senile dementia. *Lancet,* **1**:783–784.

Bowen, D. M., Allen, S. J., Benton, J. S., Goodhardt, M. J., Haan, E. A., Palmer, A. M., Sims, N. R., Smith, C. C. T., Spillane, J. A., Esiri, M. M., Neary, D., Snowdon, J. S., Wilcock, G. K., and Davison, A. N. (1983). Biochemical assessment of serotonergic and cholinergic dysfunction and cerebral atrophy in Alzheimer's disease. *J. Neurochem.* **41**:266–272.

Cross, A. J., Crow, T. J., Perry, E. K., Perry, R. H., Blessed, G., and Tomlinson, B. E. (1981). Reduced dopamine $\beta$-hydroxylase activity in Alzheimer's disease. *Br. Med. J.* **282**:93–94.

Cross, A. J., Crow, T. J., Johnson, J. A., Joseph, M. H., Perry, E. K., Perry, R. H., Blessed, G., and Tomlinson, B. E. (1983). Monoamine metabolism in senile dementia of Alzheimer type. *J. Neurol. Sci.* **60**:383–392.

Davies, P., and Maloney, A. J. F. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2**:1403.

Davies, P., Katzman, R., and Terry, R. D. (1980). Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementia. *Nature* **288**:279–280.

DeJong, R. N., and Pope, A. (1975). Complex cerebral functions. In *The Nervous System, Clinical Neurosciences, Vol. 2* (D. B. Tower and T. N. Chase, Eds.), Raven Press, New York, pp. 449–456.

Ferrier, I. N., Cross, A. J., Johnson, J. A., Roberts, G. W., Crow, T. J., Corsellis, J. A. N., Lee, Y. C., O'Shaughnessy, D., Adrian, T. E., McGregor, G. P., Baracese-Hamilton, A. J., and Bloom, S. R. (1983). Neuropeptides in Alzheimer type dementia. *J. Neurol. Sci.* **62**:159–170.

Gottfries, C. G. (1979). Amine metabolism in normal ageing and in dementia disorders. In *Biochemistry of Dementia,* (P. J. Roberts, Ed.), John Wiley and Sons, Chichester, pp. 213–234.

Gottfries, C. G. (1981). Levels of monoamines, monoamine metabolites and activity in related enzyme systems correlated to normal ageing and in patients with dementia of Alzheimer type. In *Apomorphine and Other Dopaminomimetics, Clinical Pharmacology, Vol. 2* (G. U. Corsini and G. L. Gessa, Eds.), Raven Press, New York, pp. 243–249.

Gottfries, C. G. (1990). Neurochemical aspects of dementia disorders. *Dementia* **1**:56–64.

Gottfries, C. G., Adolfsson, R., Aquilonius, S. M., Carlsson, A., Oreland, L., Svennerholm, L., and Winblad, B., (1980). Parkinsonism and dementia disorders of the Alzheimer type: Similarities and differences. In *Parkinson's Disease—Current Progress, Problems and Management* (U. K. Rhinne, M. Klingler, and G. Stamm, Eds.), Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 197–208.

Gottfries, C. G., Adolfsson, R., Aquilonius, S. M., Carlsson, A., Eckernas, S. A., Nordberg, A., Oreland, L., Svennerholm, L., Wiberg, A., and Winblad, B., (1983). Biochemical changes in dementia disorders of Alzheimer type (AD/SDAT). *Neurobiol. Aging* **4:** 261–271.

Hardy, J., Adolfsson, R., Alafuzoff, I., Bucht, G., Marcusson, J., Nyberg, P., Perdahl, E., Wester, P., and Winblad, B. (1985). Transmitter deficits in Alzheimer's disease. *Neurochem. Int.* **7:**545–563.

Ishii, T. (1966). Distribution of Alzheimer's neurofibrillary changes in the brain stem and hypothalamus of senile dementia. *Acta Neuropathol. (Berl.)* **6:**181–187.

Iversen, L. L., Rossor, M. N., Reynolds, G. P., Hills, R., Roth, M., Mountjoy, C. Q., Foote, S. L., Morrison, J. H., and Bloom, F. E. (1983). Loss of pigmented dopamine-$\beta$-hydroxylase positive cells from locus coeruleus in senile dementia of Alzheimer's type. *Neurosci. Lett.* **39:**95–100.

Mann, D. M. A., Lincoln, J., Yates, P. O., Stamp, J. E., and Toper, S. (1980). Changes in the monoamine containing neurones of the human CNS in senile dementia. *Br. J. Psychiat.* **136:**533–541.

Mann, D. M. A., Yates, P. O., and Hawkes, J. (1982). The noradrenergic system in Alzheimer and multi-infarct dementias. *J. Neurol. Neurosurg. Psychiat.* **45:**113–119.

Pearce, J. (1974). Mental changes in Parkinsonism. *Br. Med. J.* **2:**445.

Perry, E. K. (1987). Cortical neurotransmitter chemistry in Alzheimer's disease. In *Psychopharmacology: The Third Generation of Progress,* (H. Y. Meltzer, Ed.), Raven Press, New York, pp. 887–895.

Perry, E. K., Perry, R. H., Blessed, G., and Tomlinson, B. E. (1977). Necropsy evidence of central cholinergic deficits in senile dementia. *Lancet* **1:**189.

Quirion, R., Martel, J. C., Robitaille, Y., Etienne, P., Wood, P., Nair, N. P. V., and Gautheir, S. (1986). Neurotransmitter and receptor deficits in senile dementia of the Alzheimer type. *Can. J. Neurol. Sci.* **13:**503–510.

Reynolds, G. P. (1983). Increased concentrations and lateral asymmetry of amygdala dopamine in schizophrenia. *Nature* **305:**527–529.

Reynolds, G. P., Arnold, L., Rossor, M. N., Iversen, L. L., Mountjoy, C. Q., and Roth, M. (1984). Reduced binding of [$^3$H]-ketanserin to cortical 5-HT$_2$ receptors in senile dementia of the Alzheimer type. *Neurosci. Lett.* **44:**47–51.

Rossor, M. N. (1982). Dementia. *Lancet* **2:**1200–1204.

Rossor, M., and Iversen, L. L. (1986). Non-cholinergic neurotransmitter abnormalities in Alzheimer's disease. *Br. Med. Bull.* **42:**70–74.

Rossor, M. N., Emson, P. C., Mountjoy, C. Q., Roth, M., and Iversen, L. L. (1980). Reduced amounts of immunoreactive somatostatin in the temporal cortex in senile dementia of Alzheimer type. *Neurosci. Lett.* **20:**373–377.

Selkoe, D. J. (1991). The molecular pathology of Alzheimer's disease. *Neuron* **6:**487–498.

Spillane, J. A., White, P., Goodhardt, M. J., Flack, R. H. A., Bowen, D. M., and Davison, A. N. (1977). Selective vulnerability of neurons in organic dementia. *Nature* **266:**558–559.

Spokes, E. G. S. (1979). An analysis of factors influencing measurements of dopamine, noradrenaline, glutamate decarboxylase and choline acetylase in human postmortem brain tissue. *Brain* **102:**333–346.

Tomlinson, B. E., and Corsellis, J. A. N. (1984). Ageing and the dementias. In *Greenfield's Neuropathology,* 4th ed. (J. H. Adams, J. A. N. Corsellis, and L. W. Duchen, Eds.), J. Wiley, New York, pp. 951–1025.

Tomlinson, B. E., Irving, D., and Blessed, G. (1981). Cell loss in the locus coeruleus in senile dementia of Alzheimer type. *J. Neurol. Sci.* **49:**419–428.

Whitehouse, P. J., Price, D. L., Clark, A. W., Coyle, J. T., and Delong, M. R. (1981). Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* **10:**122–126.

Winblad, B., Adolfsson, R., Carlsson, A., and Gottfries, C. G. (1982). Biogenic amines in brains of patients with Alzheimer's disease. In *Alzheimer's Disease: A Report of Progress in Research, Vol. 19* (S. Corkin, K. L. Davis, J. H. Growdon, E. Usdin, and R. J. Wurtman, Eds.), Raven Press, New York, pp. 25–33.