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Alteration of Muscarinic Receptor Subtypes in CA1 field of Hippocampus in Senile Dementia of Alzheimer Type: an autoradiographic study

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ABSTRACT

This investigation confirmed decreases in muscarinic acetylcholine receptor (mAChR) concentration in the hippocampus of patients with senile dementia of Alzheimer-type (SDAT) using a binding assay with homogenate. We performed quantitative *in vitro* autoradiography of mAChR in the hippocampus obtained at autopsy from non-demented controls and SDAT patients, who had a marked memory disturbance since the early stage. ^{3}H-QNB (1.09nM) and ^{3}H-pirenzepine (18.0nM) were used as ligands to label total mAChR and M1 receptors, respectively. Total mAChR and M1 receptor densities showed a decrease by about 20% and 25%, respectively, in the CA1 area of SDAT cases, when compared with that of normal control cases. No changes were observed in the dentate gyrus, CA3, subiculum or entorhinal cortex. These observations are thought to be partly causative of the marked memory deficits which appeared in these cases.

Key words: *Senile dementia of Alzheimer type, Hippocampus, Muscarinic acetylcholine receptor, in vitro receptor autoradiography*

Senile dementia of Alzheimer-type (SDAT) is a progressive neurodegenerative disorder, characterized clinically by deficits in memory and cognition with focal neurologic signs. It is characterized pathologically by numerous senile plaques and neurofibrillary tangles especially in the cerebral cortex, amygdala and hippocampus.

Druchman and Leavitt (1974)[8] have shown that anticholinergic drugs cause a memory disturbance in healthy volunteers. Choline acetyltransferase (CAT), a presynaptic cholinergic marker, has been found to decrease in the autopsied brains of SDAT cases[2,6]. Whitehouse et al[28] have shown the selective loss of cholinergic neurons in the nucleus basalis Meynert of SDAT, which projects cholinergic fibers directly to the neocortex. Also, CAT activity has been observed in the senile plaques immunohistochemically[26]. These several observations suggest that impaired cholinergic neurons may be one of the causes of SDAT.

A number of studies have dealt with changes of muscarinic receptors (mAChR) in the SDAT brain. Most of them have shown no changes in mAChR in the cerebral cortex[7,10,20-22,24]. Nevertheless, a decrease in mAChR in the hippocampus of SDAT brains has been reported when compared with normal controls[15,19-22,24]. Recently, mAChR has been divided into subtypes according to specific ligands. Muscarinic agonist binding sites can be distinguished by three affinity states which display super high, high and low affinities for agonists[3]. There is another classification based on a high affinity antagonist for the muscarinic receptor subtypes. M1 receptor has been defined as a high affinity binding site for pirenzepine (PZ) and M2 as a low affinity binding site[9]. The relationship between the classification based on agonist and antagonist has not yet been clarified. We have adopted the latter classification in this paper. Several groups have examined changes in muscarinic receptor subtypes in the SDAT brain. Most of them have studied merely changes in (H) and (L)[15,22]. However, there have been few reports concerning M1 or M2 receptors in the SDAT brain.

MATERIALS AND METHODS

The brains used in this study are shown in Table 1. All cases had marked memory disturbance since the early SDAT and progressed to the stage of complete mental deterioration. One hemisphere from each brain was frozen immediately after removal and stored at -80°C. Another half was

Table 1. Autopsied brains used in this study.

Patient Case	Sex	Age (years)	Postmortem delay (hours)	Clinical Diagnosis	onset (age)	Cause of death
1*	M	56	13	Polymyositis		Bronchopneumonia
2*	M	75	5	Crow-Fukase Disease		Bronchopneumonia
3	M	44	9	SDAT	40	Bronchopneumonia
4	F	65	3	SDAT	59	Bronchopneumonia
5	F	87	5	SDAT	84	Bronchopneumonia

*: control, SDAT: Senile dementia of Alzheimer type

used for neuropathological investigation. The frozen hemisphere was cut into 20 μm thick coronal sections which were placed onto gelatin-chromium-coated glass slides. Appropriate sections were then processed for *in vitro* quantitative receptor autoradiography.

Brain sections were stored in the freezer until use. The hippocampal sections dissected from the whole were incubated simultaneously. To label total mAChR, two sections of the each hippocampus were incubated for 60 min at room temp. in 0.1 M phosphate buffer (pH7.4) ($NaH_2PO_4\,2H_2O$, $Na_2HPO_4\,12H_2O$) containing 1.09 nM ^{3}H-quinuclidinyl benzilate (QNB) (43.6 Ci/mmol, New England Nuclear (NEN)). For labeling M1 receptors, they were incubated for 90 min at room temp. in 50 mM Na-K phosphate buffer (pH7.4) (KH_2PO_4, $Na_2HPO_4{\cdot}12H_2O$) containing 18.0 nM ^{3}H-PZ (85.0 Ci/mmol, NEN). The non-specific binding of ^{3}H-QNB and ^{3}H-PZ was determined using the other section of each brain incubated in the presence of 1μM atropine and 1μM PZ, respectively. After the incubation, sections were rinsed in the cold buffer, for 6 and 10 min, respectively, dipped in distilled water to remove ions, and then rapidly dried with a cold-air drier. Incubated sections were subsequently placed in cassettes and exposed to a tritium-sensitive film (Sakura) with ^{3}H-standard microfilm (Amersham) for 28 or 42 days, respectively. After exposure, the films were developed, then rinsed in running water for 30 min, and dried at room temp. The developed films were quantitatively analyzed using IBAS II (Zeiss), a computerized densitometer.

RESULTS

Autoradiographs of ^{3}H-QNB binding to the total population of muscarinic receptors are shown in Fig. 1. The distribution of ^{3}H-QNB binding sites was in agreement with previous reports[4,5], and that in SDAT cases was very similar to the control cases. The receptor density was calculated in the DG, CA3, CA1, subiculum and entorhinal cortex (layers II-III and IV-VI). In the CA1 area, nearly a 20% decrease in density of ^{3}H-QNB binding sites was recognized (Fig. 2) by densitometry.

The distribution of ^{3}H-PZ binding sites (M1 receptors) was similar to that of ^{3}H-QNB binding sites, while there were few M1 receptors in the lateral geniculate body and thalamus (Fig. 3). Fig. 4 shows the M1 receptor density in the DG, CA3, CA1, subiculum and entorhinal cortex of control and SDAT cases. The M1 receptor decreased in the CA1 of SDAT brains by about 25%, when compared with the control cases.

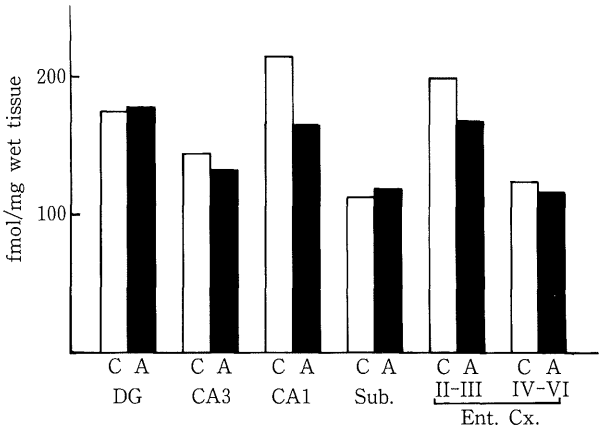

Fig. 2. Density of ^{3}H-QNB binding sites in the dentate gyrus (DG), CA3, CA1, subiculum (Sub) and entorhinal cortex (Ent. Cx.) in normal individuals (open columns) and in SDAT (hatched columns). Values are expressed as the mean of experiments.

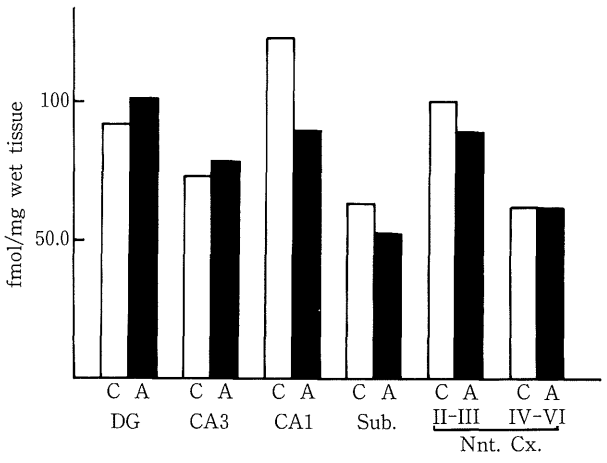

Fig. 4. Density of ^{3}H-PZ binding sites in the dentate gyrus, CA3, CA1, subiculum and entorhinal cortex in normal individuals (open columns) and SDAT (hatched columns). Values are expressed as the mean.

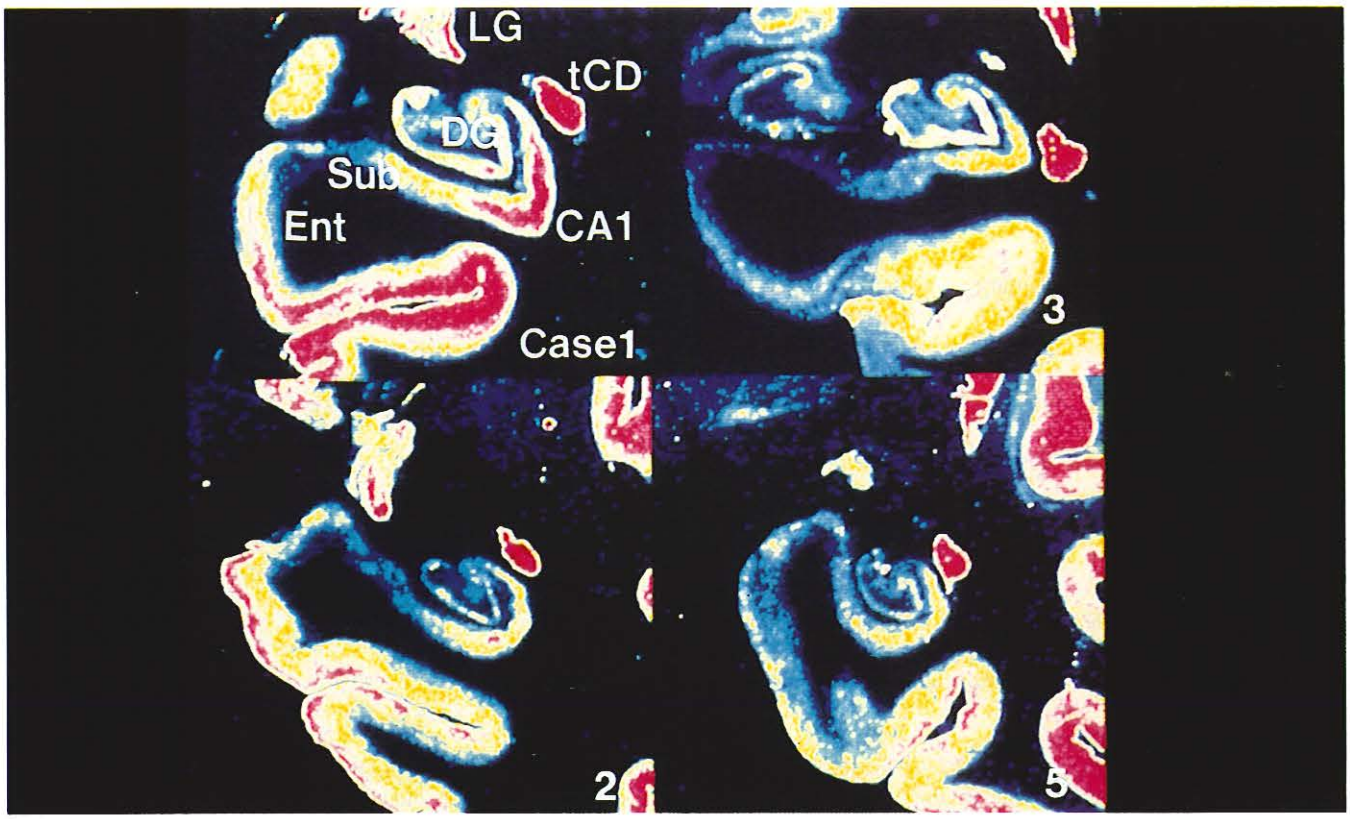

Fig. 1. Color imagings of the distribution of mAChR labeled by ^{3}H-QNB in the hippocampal regions of two control cases (case 1 and 2) and two SDAT cases (case 3 and 5). MAChR densities in the CA1 of SDAT decreased when comparaed with the control subjects. Ent: the entorhinal cortex DG: the dentate gyrus LG: the lateral geniculate body tCD: tail of the caudate Sub: the subiculum.

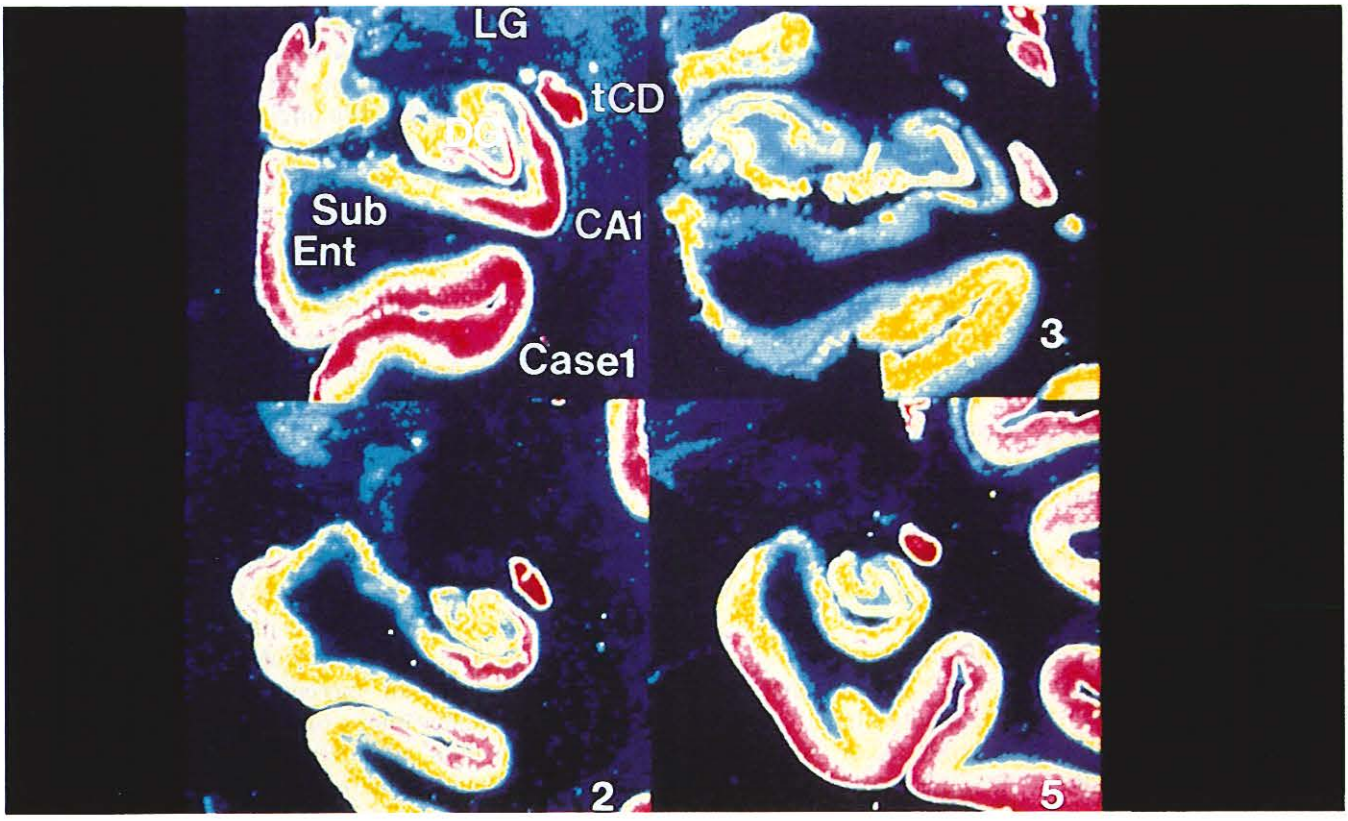

Fig. 3. Color imagings of the distribution of ^{3}H-PZ labeled M1 in the hippocampus of two control and two SDAT cases. There were low M1 receptor densities in the lateral geniculate body. M1 receptor densities in the CA1 of SDAT show a decrease compared with the control values.

Table 2. Summary of quantitative studies on changes of mAChR in the hippocampus of SDAT, using homogenate.

AUTHORS		LIGAND	CONC.	CONT.	SDAT	INH.	SUB.		
Davies & Verth	(1978)[a]	^{3}H-QNB	10nM	451	402				
Reisene et al.	(1978)[a]	^{3}H-QNB	0.14nM	553	153↓				
Rinne et al.	(1985)[b]	^{3}H-QNB	sat.	401	323↓				
Nordberg et al.	(1986)	^{3}H-QNB	0.2nM	327	243↓	CB	SH	11.0%	9.7%
							H	16.0%	18.8%
							L	74.8%	71.7%
Shimohama et al.	(1986)	^{3}H-QNB	sat.	717	469↓				
Reinikainen et al.	(1987)[a]	^{3}H-QNB	0.6nM	512	323↓				
Smith et al.	(1988)[c]	^{3}H-NMS	2.4nM	551	465	CB	H	265	225↓
							L	286	239
						P2	M1	418	332↓
							M2	191	155
Araujo et al.	(1988)	^{3}H-PZ	sat.				M1	287	358↑
		^{3}H-ACH	sat.				H	39.3	17.1↓
Rinne et al.	(1978)[b]	^{3}H-QNB	1.0nM	378.3	320.6↓	CB	H	106.7	83.6↓
							L	273	237

(fmol/mg protein)

INH.: inhibitor, SUB.: subtype of mAChR, CB: carbachol
↓ : significant decrease compared with control values.
↑ : significant increase compared with control values.

Sat: saturation experiments.
a): Number of binding sites was determined at only one concentration of radio-ligand.
b): The ratios of muscarinic receptor subtypes were obtained from inhibition curves.
c): H: The amount of ^{3}H-NMS (2×10^{-9}M) was displaced in the presence 3×10^{-4}M carbacol.
L: The residual specific binding sites were in the presence of 3×10^{-4}M carbacol.
M1: The amount of ^{3}H-NMS (2×10^{-9}M) bound multiplied by 2.
M2: The amount of ^{3}H-NMS (2.4×19^{-9}M) bound in the presence of PZ (2×10^{-6}M) multiplied by 1.6.

DISCUSSION

Recent studies on changes in mAChR in the SDAT brain have shown no changes in the cerebral cortex and decreases in the hippocampus of the SDAT brains. We can summarize thus reports describing changes of mAChR in the hippocampus of SDAT cases (Table 2). These studies can be divided into three groups according to methodology. 1) Kd and Bmax were analyzed by saturation experiments[1,21,24]. 2) The number of binding sites was obtained at a certain concentration of ^{3}H-ligand[7,19,20,25]. 3) The population of each muscarinic receptor subtype was obtained from inhibition experiments[15,22]. Davies and Verth (1978)[7] failed to show any significant decrease in mAChR in SDAT.

Two studies employed *in vitro* quantitative receptor autoradiography[11,18]. These studies used ^{3}H-n-methyl scopolamine (NMS) as a ligand and showed no significant difference in mAChR concentration of the hippocampus between control and SDAT cases. These results were in agreement with those shown by Smith et al (1988)[25], who investigated changes in mAChR of SDAT with homogenate using ^{3}H-NMS as a ligand. However, many groups have shown a decreased mAChR in the hippocampus using ^{3}H-QNB as a ligand. Probst et al (1988)[18] showed a significant decrease in mAChR density in the CA1 area of clinicaly severe cases. Whether the decrease in mAChR occurs or not seems to result from the difference in the clinical background of SDAT brains and to be dependent on the chief clinical symptoms, such as memory disturbance or other mental disorders. Another possibility is ascribable to the difference in the ligand used: NMS or QNB. We investigated changes of mAChR in the hippocampus of SDAT using ^{3}H-QNB as a ligand, and noticed a decrease in the mAChR density in the CA1 area. Since we examined brains from clinically advanced cases, our data were in accordance with those of the severe cases reported by Probst et al[18].

There have been very few reports on the changes of M1 (high affinity PZ binding sites) in the hippocampus of SDAT cases. We studied the changes of M1 in the hippocampus of SDAT using ^{3}H-PZ as a ligand and confirmed the decrease in M1 receptor density in the CA1 area. Smith et al (1988)[25] have reported on the lines of results employing similar methods, by which the M1 receptor concentration was obtained from an inhibition curve of ^{3}H-NMS (2.4 nM) with 2×10^{-6}M PZ using homogenate. On the contrary, Araujo et al (1988)[1] reported an increase in ^{3}H-PZ binding sites in the hippocampus of SDAT through saturation experiments with homogenate tissues. They speculated that the increase might be due to post-synaptic up-regulation. The difference from our results could be attributed to the difference of stage in the course of the disease.

The cholinergic fibers in the hippocampus are

mainly projected from the septum via fimbria-fornix[12,14]. Lesion of the fimbria failed to cause changes in muscarinic receptors in the rat hippocampus[16,23]. Our data along with those by Overstreet et al[16] and Sabato et al[23] suggest that the decrease in mAChR in the CA1 area of SDAT would not be produced by degeneration of cholinergic neurons in the septum, but by a postsynaptic degeneration in the hippocampus.

A positive correlation has been reported between decrease in CAT activity and memory function[17,27]. Furthermore, a bilateral lesion limited to the CA1 area of the hippocampus has been shown to cause enduring memory impairment by Zola-Morgan et al (1986)[29]. Taking these data into consideration, the decreases in mAChR in the CA1 of SDAT brains may closely correlate with the intensive memory dysfunction from the early stage of SDAT.

In conclusion, we confirmed the decrease in mAChR and M1 receptor (high affinity PZ binding sites) in the CA1 area field of the hippocampus using in vitro quantitative receptor autoradiography. Besides the degeneration of cholinergic neurons hitherto described, emphasis should be placed on the decrease in mAChR density in the CA1 area. This may be pathogenesis of marked memory disturbance during the early stage of the disease.

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REFERENCES

1. **Araujo, D.M., Lapchak, P.A., Rabitaille, Y., Gauthier, S. and Quirion, R.** 1988. Differential alteration of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. J. Neurochem. **50**: 1914–1923.
2. **Bowen, D.M., Smith, C.B., White, P. and Davison, A.N.** 1976. Neurotransmitter-related enzymes and inclices of hypoxia in senile dementia and other abiotrophies. Brain **99**: 459–496.
3. **Birdsall, N.J.M., Burgen, A.S.V. and Hulme, E.C.** 1978. The binding of agonists to brain muscarinic receptors. Mol. Pharmacol. **14**: 723–726.
4. **Cortes, R., Probst, A. and Palacios, J.M.** 1984. Quantitative light microscopic autoradiographyic localization of cholinergic muscarinic receptors in the human brain: brainstem. Neuroscience **12**: 1003–1026.
5. **Cortes, R., Probst, A., Tobler, H.J. and Palacios, J.M.** 1986. Muscarinic cholinergic receptor subtypes in the human brain. Brain Res. **362**: 239–253.
6. **Davies, P. and Malony, A.J.F.** 1976. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet **ii**: 1403.
7. **Davies, P. and Verth, A.H.** 1978. Regional distribution of muscarinic acetylcholine receptor in normal and Alzheimer's-type dementia brains. Brain Res. **138**: 385–392.
8. **Drachman, D.A. and Leavitt, J.** 1974. Human memory and the cholinergic system. a relationship to aging? Arch. Neurol. **30**: 113–121.
9. **Hammer, R., Berrie, C.P., Birdsall, N.J.M., Burgen, A.S.V. and Hulme, E.C.** 1983. Pirenzepine distinguishes between different subclasses of muscarinic receptors. Nature **283**: 90–92.
10. **Kellar, K.J., Whitehouse, P.J., Martino-Barrows, A.M., Marcus, K. and Price, D.L.** 1986. Muscarinic and nicotinic cholinergic binding sites in Alzheimer's disease cerebral cortex. Brain Res. **436**: 62–68.
11. **Lang, W. and Henke, H.** 1983. Cholinergic receptor binding and autoradiography in brains of non-neurological and senile dementia of Alzheimer-type patients. Brain Res. **267**: 271–280.
12. **Lewis, P.R. and Shute, C.C.D.** 1967. The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfonical organ and supra-optic crest. Brain **90**: 521–540.
13. **Mash, D.C., Flynn, D.D. and Poter, L.T.** 1985. Loss of M2 muscarinic receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic dennervation. Science **228**: 1115–1117.
14. **Mosko, S., Lynch, G. and Cotman, C.W.** 1973. The distribution of septal projection to the hippocampus of the rat. J. Comp. Neurol. **152**: 163–174.
15. **Norderg, A., Alafuzoff, I. and Winblad, B.** 1986. Muscarinic receptor subtypes in hippocampus in Alzheimer's disease and mixed dementia type. Neurosci. Let. : 160–164.
16. **Overstreet, D.H., Speth, R.C., Hruska, R.E., Ehlert, F., Dumont, Y. and Yamamura, H.I.** 1980. Failure of septal lesions to alter muscarinic cholinergic or benzodiazepane binding sites in hippocampus of rat brain. Brain Res. **195**: 203–207.
17. **Perry, E.K., Tomlinson, B.E., Blessed, G., Bergmann, K., Gibson, P.H. and Perry, R.H.** 1978. Correlation of cholinergic abnormalities with senile plaques and mental test sccores in senile dementia. Brit. Med. J. **2**: 1457–1459.
18. **Probst, A., Cortes, R., Ulrich, J. and Palacios, M.** 1988. Differential modification of muscarinic cholinergic receptors in the hippocampus of patients with Alzheimer's disease: an autoradiographic study. Brain Res. **450**: 190–201.
19. **Reinikainen, K.J., Riekkinen, P.J., Halonen, T. and Laakso, M.** 1987. Decreased muscarinic receptor binding in cerebral cortex and hippocampus in Alzheimer's disease. Life Sci. **41**: 453–461.
20. **Reisine, T.D., Yamamura, H.I., Bird, E.D., Spokes, E. and Enna, S.J.** 1978. Pre- and postsynaptic neurochemical alterations in Alzheimer's disease. Brain Res. **159**: 477–481.
21. **Rinne, J.O., Laakso, K., Lonnberg, P., Molsa, P., Paljarvi, L., Rinne, J.K., Sako, E. and Rinne, U.K.** 1985. Brain muscarinic receptors in senile dementia. Brain Res. **336**: 19–25.
22. **Rinne, J.O., Lonnberg, P., Majamaki, P. and Rinne, U.K.** 1989. Brain muscarinic receptor subtypes are differently affected in Alzheimer's disease and Parkinson's disease. Brain Res. **483**: 402–406.
23. **Sabato, U.C., Aguilar, J.S., Medina, J.H. and De Robertis.** 1981. Changes in rat hippocampal benzodiazepine receptors and lack of changes in muscarinic receptors after fimbria-fornix lesions. Neurosci. Let. **27**: 193–197.
24. **Shimohama, S., Taniguchi, T., Fujiwara, M. and**

Kameyama, M. 1986. Changes in nicotinic and muscarinic cholinergic receptors in Alzheimer-type dementia. J. Neurochem. **46**: 288–293.

25. **Smith, C.J., Perry, E.K., Candy, J.M., Johnson, M., Bonham, J.R., Dick, D.J., Fairbairn, A., Blessed, G. and Birdsall, N.J.M.** 1988. Muscarinic cholinergic receptor subtypes in hippocampus in human cognitive disorders. J. Neurochem. **50**: 847–856.
26. **Struble, R.G., Hedreen, J.C., Cork, L.C. and Price, D.L.** 1982. Cholinergicinnervation in neuritic plaques. Science. **216**: 413–415.
27. **Wilcock, G.K., Esiri, M.M., Bowen, D.M. and Smith, C.C.T.** 1982. Alzheimer's disease. Correlation of cortical choline acetyltrans ferase activity with the severity of dementia and histological abnormalities. J. Neuro. Sci. **57**: 407–417.
28. **Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T. and Delong, M.R.** 1981. Alzheimer's disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann. Neurol. **10**: 122-126.
29. **Zola-Morgan, S., Squire, L.R. and Amaral, D.G.** 1986. Human amnesia and the medial temporal region: enduring memory impairement following a bilateral lesion limited to field CA1 of the hippocampus. J. Neurosci. **6**: 2950–2967.