Acta Medica Okayama

Volume 50, *Issue* 4	1996	*Article* 1

AUGUST 1996

Clinical applications of neurotransmitter-receptor studies in geriatric neuropharmacology.

Marvin Gomez-Vargas*   Norio Ogawa†

*Okayama University,
†Okayama University,

Clinical applications of neurotransmitter-receptor studies in geriatric neuropharmacology.*

Marvin Gomez-Vargas and Norio Ogawa

Abstract

The use of ligand-binding methods to study neurotransmitter-receptor sites has made its impact on almost all aspects of biological pursuits including research on aging and neurodegenerative diseases. In the past, most of the research in biochemical gerontology has largely centered around changes in various neurotransmitters and enzymatic activities. The molecular basis of aging and neurodegeneration at the level of neurotransmitter-receptor interactions has been highly appreciated in the last two decades as a result of receptor binding studies. It is now possible to obtain information about the regional distribution of neurotransmitter receptors in the brain, the pharmacological and biochemical characteristics of these sites, and the functional interrelationships between different neuronal systems in normal and pathological conditions. The passage of time after maturity is accompanied by measurable physiologic decline in virtually all systems. It is the aim of this work to discuss the practical aspects of neurotransmitter and/or drug (ligand)-receptor binding studies, highlighting some examples of their applications to geriatric neuropharmacology research, with special consideration to learning impairment and memory loss in normal and in pathological aging processes.

KEYWORDS: aging, neurodegenerative diseases, neurotransmitters, receptor binding, neuropharmacology

---

Review

Clinical Applications of Neurotransmitter-Receptor Studies in Geriatric Neuropharmacology

Marvin Gómez-Vargas* and Norio Ogawa

Department of Neuroscience, Institute of Molecular and Cellular Medicine, Okayama University Medical School, Okayama 700, Japan

The use of ligand-binding methods to study neurotransmitter-receptor sites has made its impact on almost all aspects of biological pursuits including research on aging and neurodegenerative diseases. In the past, most of the research in biochemical gerontology has largely centered around changes in various neurotransmitters and enzymatic activities. The molecular basis of aging and neurodegeneration at the level of neurotransmitter-receptor interactions has been highly appreciated in the last two decades as a result of receptor binding studies. It is now possible to obtain information about the regional distribution of neurotransmitter receptors in the brain, the pharmacological and biochemical characteristics of these sites, and the functional interrelationships between different neuronal systems in normal and pathological conditions. The passage of time after maturity is accompanied by measurable physiologic decline in virtually all systems. It is the aim of this work to discuss the practical aspects of neurotransmitter and/or drug (ligand)-receptor binding studies, highlighting some examples of their applications to geriatric neuropharmacology research, with special consideration to learning impairment and memory loss in normal and in pathological aging processes.

Key words: aging, neurodegenerative diseases, neurotransmitters, receptor binding, neuropharmacology

N eurotransmitters can be broadly defined as substances that, on release from nerve terminals, act on specific sites at postsynaptic membranes to produce either excitation or inhibition of the target cell. These specific sites, where the first interactions between neurotransmitters and target tissues take place, are called receptors. Neurotransmitter receptors are proteins with complex structures for both binding the transmitter and transducing this binding into a biologically relevant effect.

Neurotransmitters are usually classified into amino acids, biogenic amines and neuropeptides. These substances have been studied together with their receptors, and the knowledge of them has greatly increased in the past 20 years, especially in the case of amines and amino acids. However, such is not the case with the information on neuropeptides and, despite the rapid advances in their research, there are limited examples in which the physiological role of a particular peptide in an identified neural pathway can be discussed exactly. Biogenic amines are 1,000 times more concentrated, and amino acids 100,000 times more concentrated than neuropeptides in the central nervous system (CNS) (1). Monoamine and peptide coexistence in the same neurons is now regarded as commonplace, and a large number of different combinations of peptides with monoamine and/or amino acid neurotransmitters has been described. This gives an idea of the interrelations and complexities encountered in neural systems, and also supports the fact that neuropeptides play important roles as neurotransmitters or neuromodulators in the CNS, given that they have biological activities even at very low concentrations. Every year, much information is produced by research studies on the effects of CNS-acting drugs on neurotransmitters and their receptors, since substances that can enhance or normalize neurotransmitter or receptor functions are

* To whom correspondence should be addressed.

regarded as highly promising effective drugs for the treatment of various neurological and psychiatric disorders.

The central role of receptors in mediating many physiological events has emerged as one of the basic tenets of modern neuropharmacology. CNS-acting drugs are now known to exert their effects via multiple routes of action, and do not depend on a single action mechanism (2). The effects of CNS-acting drugs have been evaluated with regard to their actions mostly on brain biogenic amine and/or amino acid neural systems, with less work being done on their relation to neuropeptides and their receptors.

Based on the assumption that some CNS-acting drugs exerted their effects through their actions on biogenic amine systems, major tranquilizers and tricyclic antidepressants, for example, were narrowly considered to "block dopamine receptors" or to "inhibit catecholamine or serotonin re-uptake", respectively. However, with the advancement of the study of receptors, CNS-acting drugs have been demonstrated to act directly on many different types of receptors (3). Major tranquilizers are now known to act, fundamentally, on alfa-1 adrenergic receptors and histamine receptors, with some actions also on serotonin, muscarinic acetylcholine and enkephalin receptors; tricyclic antidepressants, on the other hand, are known for their anti-cholinergic actions as well as their effects on 5-HT$_2$, alfa-2 adrenergic, enkephalin and thyrotropin-releasing hormone (TRH) receptors (4).

In recent years, studies at the gene level by methods of recombinant DNA technology have been applied to existing research efforts in receptor studies. Several groups have cloned genes for various receptors and three-dimensional models of some of these receptors have been developed based on the putative amino acid sequence. It seems that the number of studies at the very detailed molecular level has increased at the expense of receptor binding studies. There is no doubt that this shift in emphasis is important in the effort to elucidate the functions of the nervous system and has placed cloning techniques at the forefront of molecular receptor research. Nevertheless, receptor binding studies must still be regarded as being of prime importance in the study of neuropharmacology since neuroscientists, through very simple, cost-effective and reliable procedures, can obtain new insights into the identification, structure-function relationship and screening of endogenous substances or clinically beneficial drugs.

Parallel Determination of Neurotransmitters and their Receptors

To gain insight into the various neurological and psychiatric diseases, the parallel measurement of both neurotransmitter and receptor levels is considered the best means of describing the action mechanisms of CNS-acting drugs. Even though the first interaction between the neurotransmitter and its target tissues occurs at the receptor, the functional mechanisms of the CNS and the evaluation of CNS-acting drugs have been studied mainly with reference to changes in the concentration of neurotransmitters only. Studies on neuropeptides, for example, have been limited to measurement of their concentrations by radioimmunoassay (RIA). However, the measurable concentration of a neurotransmitter is the sum of the substance being synthesized, stored, and released, and a large storage capacity may conceal fluctuations of minute released amounts of what are sometimes extremely potent biological peptides. Increases in neurotransmitter levels do not necessarily coincide with enhanced function, since cessation of release could cause such increases. The functions of neurons subserved by amines may be evaluated by simultaneously measuring the concentration of amines and their metabolites, but the neural function mediated by peptides, on the other hand, cannot be accurately estimated by RIA because peptide precursors, from the neuropeptide synthesis, are unavoidably included in the measurements. The distinctive features of monoamine and neuropeptide synthesis are represented in Fig. 1.

With the advent of biochemical techniques to measure the presence of receptor sites in the brain (5), it has become possible to obtain new information about the interaction of CNS neurotransmitters and their receptors. Receptors localized at the synaptic junctions are not static entities and their binding activity is considered to change in closer association with the condition of the synapse, particularly with alterations in transmitter release and/or synthesis. Continued stimulation of receptors with agonists, due to increases in transmitter release and/or synthesis, results in a state of "down-regulation" (also referred to as refractoriness or desensitization). Clearly, receptor down-regulation must be taken into account when interpreting binding data in intact cell systems. Regulation of the binding activity of receptors provides an intriguing mechanism for the control of cell responsive-

ness. Based in normal conditions (equilibrium) it is possible to predict the consequences of potent and long-term stimulation of receptors (Fig. 2). Predictably, the inverse condition, up-regulation (also known as supersensitivity), is frequently observed following a continuous reduction in receptor stimulation. Mostly, up-regulation results from genetically regulated *de novo* synthesis of additional receptors induced by hormones or other cell regulators (Fig. 2).

The effects of some pharmacological agents on peptide-mediated neural systems can become clear only after measurements of the receptor levels (6). Therefore, for accurate assessment of neural function in these sys-

tems, the neuropeptide levels must be measured along with the receptor levels. This parallel determination of both peptide and receptor levels is not only complementary but indispensable for proper evaluation of synaptic activities.
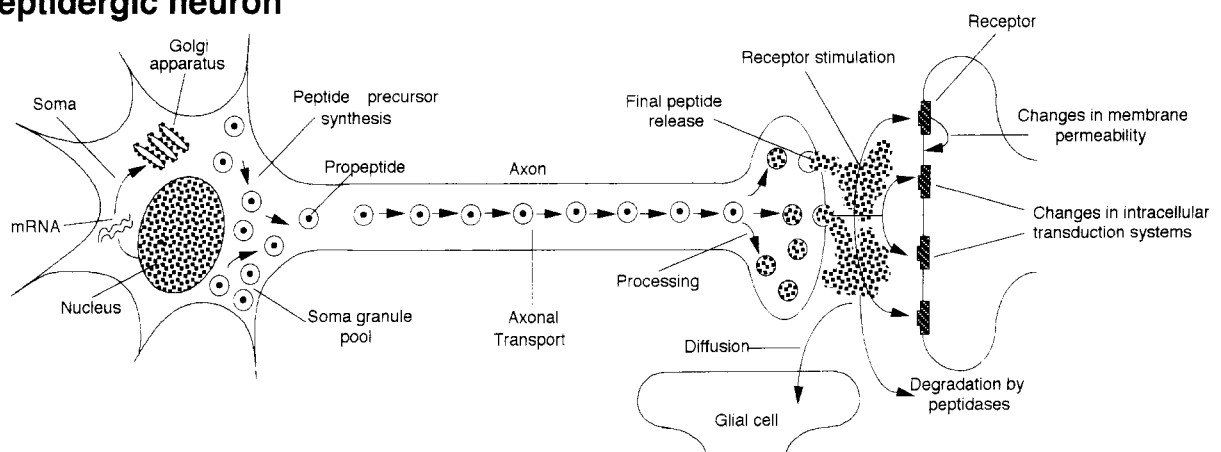
Applications of Drug Receptor Studies to Geriatric Neuropharmacology

The degenerative processes of the CNS are characterized by the selective loss of neurons. We consider the different neurodegenerative diseases as the different

a) Aminergic neuron
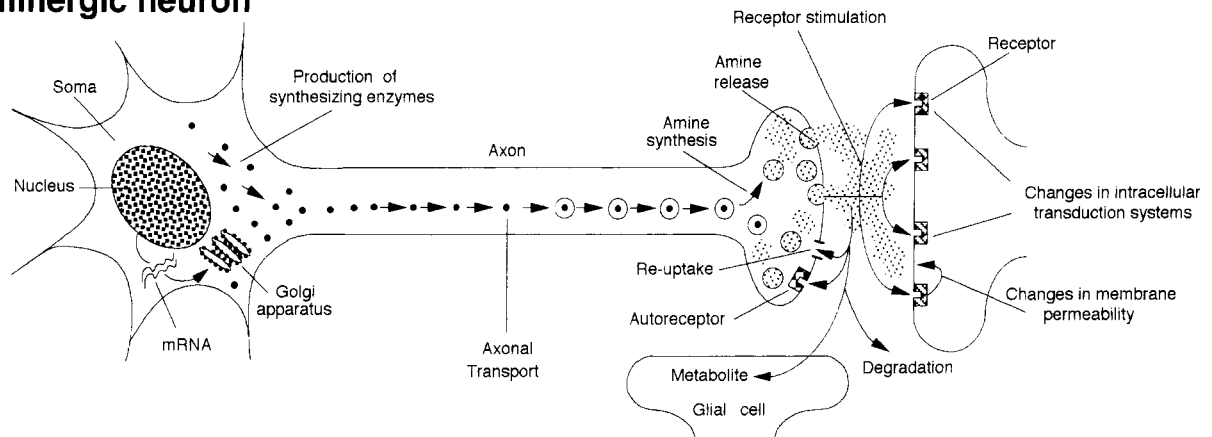
b) Peptidergic neuron

Fig. 1   Differences in the synthesis, storage and release of neurotransmitters by aminergic and peptidergic neurons. a) Amine synthesizing enzymes are composed in the soma and then transported to the nerve terminal where amines are synthesized and stored until release. After release, a considerable part of these amines can be taken up to the nerve terminals by active reuptake sites. b) Peptide synthesis is directed by mRNA on ribosomes, and thus takes place only in the cell soma; all peptides are synthesized as much larger precursors (propeptides) from which the peptide is cleaved by special processing peptidases, stored into vesicles and transported down to the nerve terminal for release; enzymatic degradation appears to be the principal mechanism for inactivation.

results of similar processes leading to neuronal death. In this section, we review only some of these conditions with special consideration to memory systems in normal and pathological aging. During normal aging, a gradual decrease in the activity of neurotransmission systems has been observed. The cholinergic system seems to show the most important alterations during aging and this is possibly associated to age-related memory loss. Therefore, we focus our attention on neurotransmitter and receptor studies in the cholinergic system deficits and believe that many of the principles probably apply to other neurotransmitters. Other important biological aspects of the aging process such as changes in other neurotransmission systems, changes in transduction systems, changes in growth response, changes in oxidative metabolism or calcium dishomeostasis are briefly mentioned or not included here because they are outside the scope of this review.

Nevertheless, caution should be exercised before assuming that any single neurotransmitter or second messenger system is the only crucial intermediate that links human behavior and molecular neurobiology in the aging population, More likely, all of the neurotransmitter systems are interwoven in a complex, interdependent web.

Learning Ability and Memory

There are diverse approaches to the biochemical basis of learning and memory. The acetylcholine (ACh)-mediated neuronal system is known to play an important role in higher cognitive functions, especially memory and learning (7, 8). Pharmacological experiments in humans and monkeys have demonstrated that decreased cholinergic transmission in the CNS results in impaired memory (9, 10). Furthermore, postmortem studies have revealed a consistent and dramatic loss of hippocampal and cortical cholinergic pathways in patients with senile dementia (11
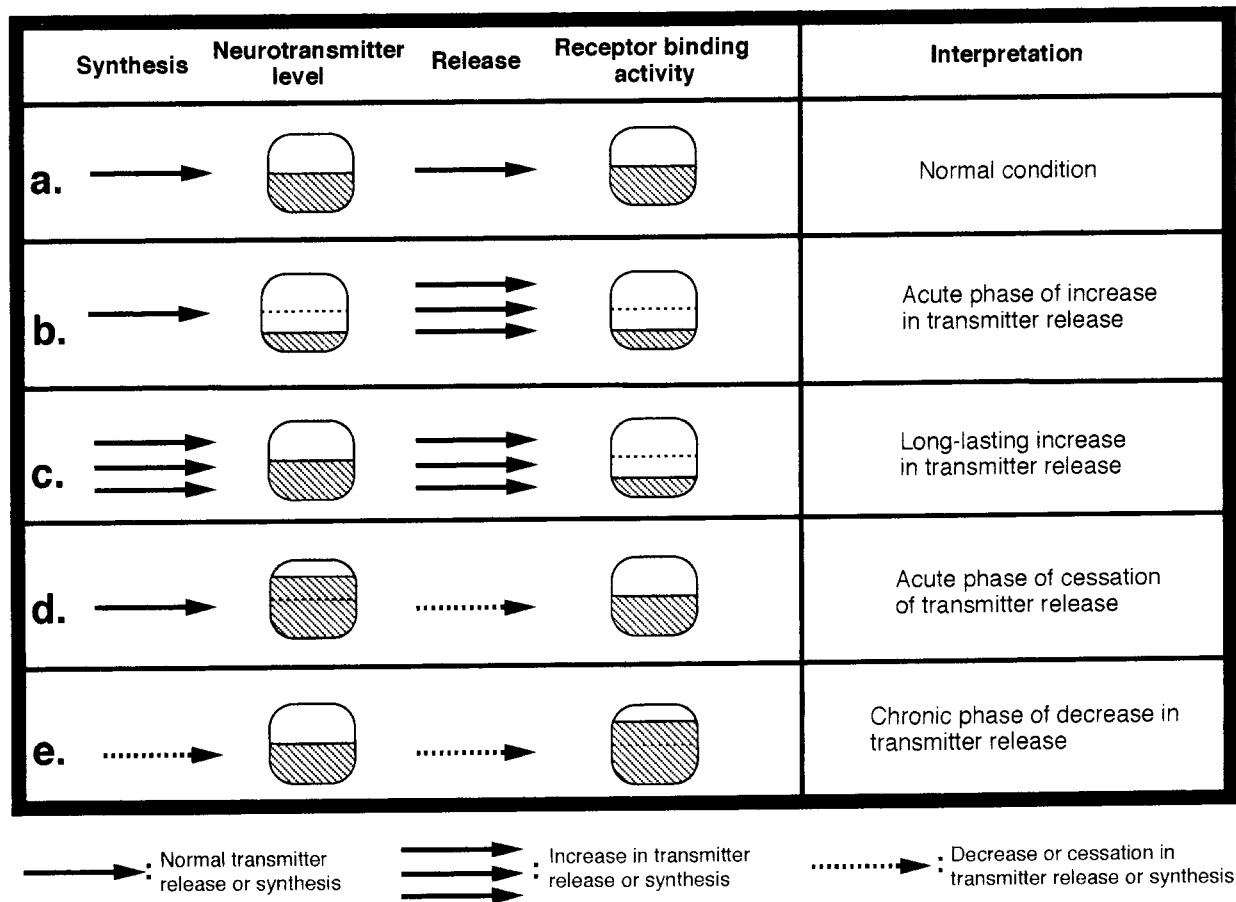

Synthesis	Neurotransmitter level	Release	Receptor binding activity	Interpretation
a.				Normal condition
b.				Acute phase of increase in transmitter release
c.				Long-lasting increase in transmitter release
d.				Acute phase of cessation of transmitter release
e.				Chronic phase of decrease in transmitter release

Normal transmitter release or synthesis

Increase in transmitter release or synthesis

Decrease or cessation in transmitter release or synthesis

Fig. 2   Regulatory and homeostatic control of receptors: effects of acute and chronic changes in agonist stimulation.

-13).

Drugs with actions on cholinergic receptors are useful tools in behavioral tests that are part of memory research in animals (14-16). The anticholinergic alkaloid, scopolamine, is one of such widely used drugs. It is well established that scopolamine decreases the function of the muscarinic neuronal system in the CNS. Many reports document that this drug also delays the acquisition of avoidance learning in rodents (14, 15, 18-19).

Drugs with antianoxic effects like bifemelane, a cerebral metabolic activator, have been reported to inhibit the age-related decreases in muscarinic and N-methyl-D-aspartate (NMDA) receptors (20, 21), and to prevent the decrease in ACh caused by brain ischemia (22). Another of such drugs is indeloxazine, which is thought to possess antianoxic effects (23), antireserpine action without anticholinergic activity (24) and facilitatory effects on learning behavior (25). In some studies, these agents were reported to improve scopolamine-induced amnesia in experimental animals trained by a conditioned avoidance procedure (25-27). Posterior studies, using the eight-arm radial maze task, reported similar results (28, 29). This task, developed by Olton and Samuelson (30), was initially devised for studying spatial memory, working memory and reference memory in rats. Many reports have shown that performance in this task is profoundly impaired by damage of the cholinergic neuronal system such as intraperitoneal injection of scopolamine (18, 19, 31), intracerebroventricular administration of the presynaptic cholinergic neurotoxin AF64A (32, 33) or hippocampal lesions (34). Higashida and Ogawa (28) evaluated the differences in the effects of scopolamine on radial maze performance in three strains of rats and concluded that scopolamine affects not only the working memory but also the motivational factor. It is well established that the cholinergic nervous systems in the CNS spread mainly in three pathways in the rat: from the septum to cerebral cortex, hippocampus and habenula; from nucleus basalis of Meynert to the frontal cortex and amygdala; and interneurons in the striatum. By the intraperitoneal injection of scopolamine, not only the septo-hippocampal pathways but also all these pathways would be depressed. The depressed pathways may contain the systems which mediate motivational factors or the ability to memorize.

In another study, the cerebral muscarinic cholinergic receptor level in aged rats, which is reduced with aging, was restored to the level of normal mature rats by the administration of bifemelane (29). Bifemelane is consid-

ered to have a potent activating effect on both the presynaptic and the postsynaptic cholinergic neuronal systems. These results suggested that the alleviation of the scopolamine-induced impairment of radial maze task performance observed in animals given bifemelane is at least partly explained by this drug's activating effect on the cholinergic neuronal systems. From the improvement in the memory-learning performance in animal models and from the biochemical findings of the effects on the cholinergic neuronal systems, bifemelane is a promising drug for the treatment of memory loss and cognitive dysfunction associated with aging, dementia and cerebrovascular disease.

A newer agent that has shown important effects on hippocampal ACh overflow in rats is linopirdine (Dup 996). Linopirdine represents a novel class of compounds which enhance depolarization-activated release of ACh. In several cognitive behavioral tests, linopirdine ameliorated the atropine-induced deficits in cognition-impaired rats (35, 36).

Selective alteration in the regulation of putative M_2 subtype muscarinic receptors (M_2-R) is apparent particularly in aged cognitively impaired rats (37). An increase in the number of M_2 binding sites could lead to a decrease in the capacity to release ACh, as recent studies have shown that M_2-R antagonists have a facilitatory ACh release and improved spatial memory in aged rats (38, 39), In one of these studies BIBN-99, an M_2-R antagonist, reversed the impairment of ACh release as well as the cognititve deficits observed in aged memory-impaired rats, and similarly, reversed scopolamine-induced amnesia in young rats (39). The efficacy of M_2-R antagonists is likely related to the blocking properties on M_2-R that are believed to act as negative autoreceptors. These findings support the idea that ACh is involved in learning and memory, and may have implications for the treatment of cognitive decline in degenerative disorders.

It is interesting that other agents may provide new and different approaches to cholinergic dysfunction in memory disorders. One class of such agents are the nerve growth factor (NGF) synthesis stimulators. NGF plays an important role in the survival and maintenance of cholinergic neurons but it can be used for medical treatment only when injected directly into the brain because it does not cross the blood-brain barrier and is easily metabolized by peptidases if administered peripherally. In one report, the NGF synthesis stimulators idebenone and propentofylline produced a significant recovery of NGF content in the

frontal cortices of aged rats and also improved performance in behavioral tests (40). Furthermore, acetyl-L-carnitine, a chemical substance able to prevent some degenerative events associated with aging, abolishes the age-associated reduction of NGF receptor (p75 NGFR) mRNA levels in the basal forebrain of old animals and mantained these levels in the cerebellum of old animals at levels almost identical to those in young control animals (41). These results suggest that the neuroprotective effect of acetyl-L-carnitine on cholinergic neurons may be exerted at the level of transcription of p75 NGFR and that restoration of these receptor levels can increase trophic support by NGF on cholinergic neurons implicated in the cognitive decline associated with aging.

A novel means of boosting central cholinergic function to overcome memory disorders is direct activation of serotoninergic 5-HT$_4$ subtype receptors (5-HT$_4$-R) by agonists. The 5-HT$_4$-R agonists BIMU 1 and BIMU 8, administered intracerebroventricularly, facilitate ACh release in the frontal cortex, but not in the striatum or hippocampus (42).

Another "indirect" approach to cholinergic activity is the case of the selective sigma ligand JO 1784. It has been demonstrated that JO 1784 is effective in reversing scopolamine-induced amnesia in the rat (43). The hippocampus is a brain area pivotally involved in learning and memory processes (44) and which has high cholinergic neuronal input. Thus, destruction of muscarinic receptors in this area can lead to profound memory deficits (45). Trimethyltin (TMT) is a potent neurotoxic organotin compound which can contribute to the loss of muscarinic receptors (45, 46). In an autoradiographic study, long-term JO 1784 administration had a "neuroprotective effect" on both M$_1$ and M$_2$ receptor subtypes in TMT-treated rats. Both receptor subtypes showed significant increases in their densities in several brain regions with the most marked effects occuring in the cortex, olfactory regions, thalamus and basal forebrain nuclei for M$_1$-R, and in the cortex, hippocampus, amygdaloid nuclei, basal ganglia and hypothalamus for M$_2$-R (47). The ability of JO 1784 to attenuate the loss of muscarinic receptors in TMT-treated animals could be of importance in the development of similar novel neuroprotective drugs.

Aging

Aging can be defined as the accumulation of changes responsible for the irreversible alteration of cells or organs that increase permanently the chance of disease and death. Some of the most devastating neurodegenerative diseases

are age-related and brain aging can influence the overall time course of neuropathological changes associated with disorders like Alzheimer's disease (AD), Parkinson's disease and vascular dementia.

Recently, along with an increase in the proportion of elderly in the population in industrialized countries, the number of aged patients with decreased mental and neurological functions, especially acquisition and memory retention, has been increasing. Senile dementia has become an increasingly important medical and social problem. The word dementia denotes a progressive decline in mental function, in memory and in acquired intellectual skills. Dementia can result from many causes and therefore is not, by itself, diagnostic of a specific disease. It is not an inevitable consequence of aging and is definitely age-related. Usually, dementia is classified into two main types: Alzheimer-type dementia (ATD) (discussed in the next section) and the cerebrovascular type.

Before answering the question of how aging is a major risk factor for neurodegenerative diseases, it is necessary to examine the main biochemical characteristic (cholinergic activity reduction) of normal aging compared with pathological conditions such as ATD (Fig. 3).

In a review of cholinergic function in aging and ATD, Bartus et al. (7) proposed the cholinergic hypothesis of geriatric intellectual dysfunction. They argued that cholinergic blockade impairs intellectual function in animal models.

Most studies of cholinergic function have involved ATD, and there have been few reports about cholinergic function in normal aging, even though disturbance of memory also occurs in normal aged people. There are differences of opinion with regard to precholinergic and postcholinergic indices in normal aging (48, 49). In several reports, the ACh concentration, a presynaptic marker, did not change significantly in normal aging (50, 51). However, Tanaka et al. (52) reported that the ACh concentration in senescent rats was markedly reduced in the frontal cortex, hippocampus, striatum and thalamus + midbrain. Although an aging-induced decrease of ACh concentration was anticipated, there are few reports on aging-induced ACh depletion because acetylcholinesterase (AChE), which is an ACh-degrading enzyme, decomposes ACh immediately and hinders the measurement of ACh concentrations. Although choline acetyltransferase (CAT) activity has been reported to be reduced (53, 54), or not significantly changed by normal

aging (55, 56), CAT activity in all four brain regions, evaluated by Tanaka *et al.* (52), was decreased. Based on these findings, the generalization that both ACh concentration and CAT activity as presynaptic cholinergic markers are reduced by normal aging, is valid. However AChE activity is not significantly changed in the senescent rat brain, as several reports have shown (57–59).

Other researchers have reported aging-induced reductions, especially in the cerebral cortex, in the density of brain quinuclidinyl phenyl-4-benzilate (QNB)-binding muscarinic cholinergic receptors (MCR), without any changes in affinity (55, 56). Changes in muscarinic M_1 subtype receptor (M_1-R) have rarely been reported. Some authors reported that the B_{max} of all regional binding sites of M_1-R, a postsynaptic cholinergic marker, was decreased in the senescent brain (52). These findings suggest that the aging-induced alterations of cholinergic indices involve the entire synapse, both the presynaptic nerve ending and the post-junctional cell (Fig. 3).

In contrast, in studies of [^{3}H]hemicholinium binding to high affinity choline uptake sites or [^{3}H]pirenzepine binding to M_1-R, Smith *et al.* (49) did not detect significant changes, but found strong correlations between

behavioral performance of aged rats and the density of these sites in the hippocampus and dentate gyrus. These findings stress the importance of combining behavioral assesment with quantitative autoradiographic studies.

It is important to highlight the effects of some drugs in the age-induced reductions of neurotransmitter and receptor levels. In one report, long-term administration (1 mg/kg i.p. for 14 days) of codergocrine mesylate (dihydroergotoxine), an ergot alkaloid, normalized CAT content and recovered MCR binding in the cerebral cortex and hippocampus of aged rats. It is interesting that repeated administration of dihydroergotoxine seemed to have no effect on the brain of young adult rats (60). In a similar study, the same authors found that another ergot alkaloid, nicergoline, specifically corrected the reduced CAT activity and MCR observed in aged rat brains, particularly in the cerebral cortex and hippocampus (61).

Not only ergot alkaloids, but also other chemical CNS-acting agents can produce similar effects on the cholinergic system. Following intraperitoneal injection of bifemelane at a daily dose of 15 mg/kg for 14 days, MCR binding in aged rat brains showed significant recovery in the cerebral cortex and hippocampus. As it was the case
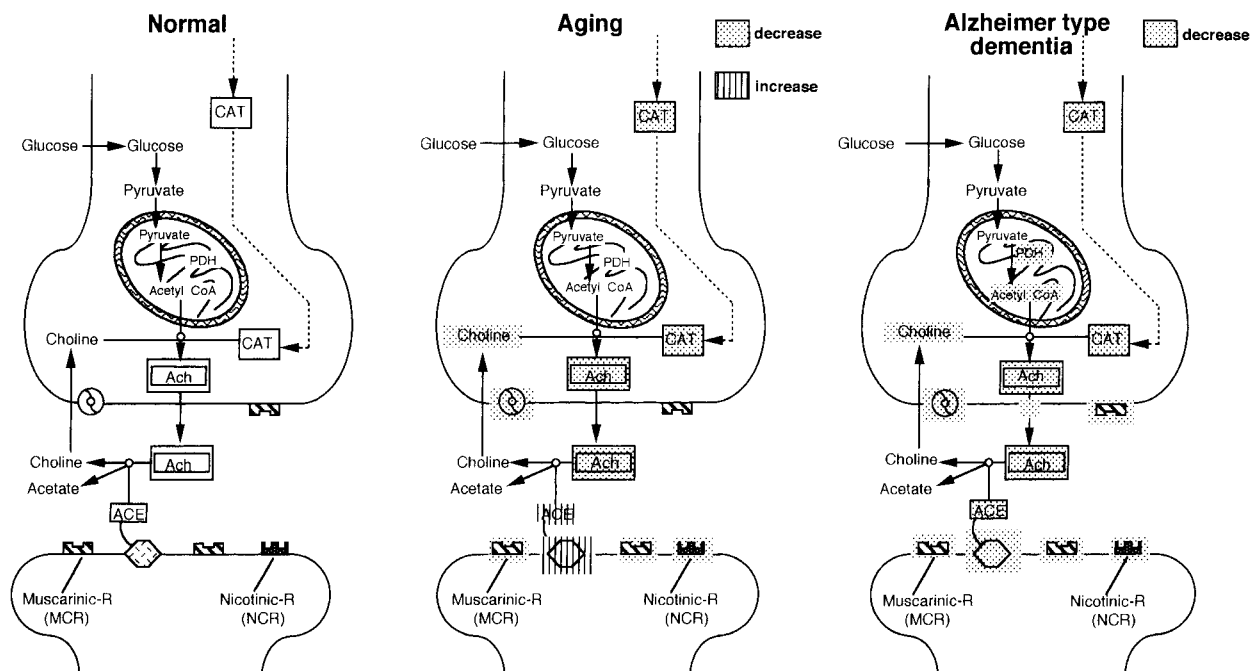
Fig. 3 Alterations in pre- and post-synaptic cholinergic markers in normal and pathological aging processes.
CAT: choline acetyltransferase; Ach: acetylcholine; ACE: acetylcholinesterase.

with dihydroergotoxine, bifemelane had no effect on MCR of young adult brains. One study (62) reported that rolipram, a cAMP-specific phosphodiesterase inhibitor, (0.1 mg/kg i.p. for 14 days) also showed ameliorating effects on the CAT activity and the M_1-R binding in the frontal cortex and the hippocampus of aged rat brains.

As we have seen, specific and reproducible changes involving the muscarinic cholinergic system have been described in both the aging rodent models and in the human nervous system. Nevertheless, relatively little information is available on changes in nicotinic cholinergic receptors occurring in normal aging, and there have been few attempts to correlate alterations in receptor densities with changes in nicotinic actions. In a quantitative auto-radiographic study, Schulz et al. observed a nearly 80 % reduction with age in the subpopulation of nicotinic receptors in several brain regions (63) and speculated that decrements in this receptors, or in the neurons expressing them, may contribute to some of the functional declines that take place during aging. This is supported by results showing cognition-enhancing abilities in aged rats with long-term administration of nicotine and synthetic nicotinic receptor agonists. One of such agonists, GTS-21, an anabasine derivative, has cognition-enhancing effects in aged rats that are comparable to those of nicotine (64) but, since GTS-21 acts preferentially on brain nicotinic receptors and is less toxic than nicotine, it may be of substantial therapeutic value in the treatment of age-associated memory impairment and/or ATD.

Receptors for excitatory amino acid neurotransmitters are classified into at least three categories: receptors sensitive to NMDA, quisqualate (QA) and kainate (KA). Among the multiple excitatory amino acids and their dependent systems, the glutamate-NMDA receptor system is believed to play a primary role in memory and learning. Greenamyre et al. (65) have shown that there are fewer NMDA receptors in the hippocampus of patients with AD. Using computer assisted image analysis, other investigators have shown a decrease in the number of NMDA receptors in aged rats in nearly all areas of the brain, but especially in the cerebral cortex and hippocampus (20).

In addition to its effects on MCR, bifemelane enhances NMDA receptors, especially in the temporal cortex, amygdaloid nucleus and hippocampus (20). These results indicate that dysfunction in learning and memory in the aged rats, as well as the therapeutic efficacy of bifemelane is due, at least in part, to the alterations of

NMDA receptors in these brain areas.

In a quantitative autoradiographic study in human post-mortem hippocampus, memantine, a known antispastic agent, inhibited the binding of the non-competitive NMDA receptor-antagonist $[^3H]$MK-801 (66). This suggests that antagonism of endogenous glutamate at limbic NMDA receptors may be a molecular mechanism by which this drug can be proposed as a neuroprotective agent.

The role of inhibitory amino acids has also gained attention in recent years. Some authors had formulated a benzodiazepine (BDZ)/GABAergic hypothesis of brain aging (67) based on positive results obtained with the utilization of flumazenil, a BDZ receptor antagonist. This drug significantly protected against age-related loss of cognitive functions in rats. It has been postulated that the age-related alterations in brain function may be attributable to the negative metabolic/trophic actions of "endogenous" BDZ ligands and/or those ingested with food.

In concordance with this hypothesis, significant correlations were recently reported in age-related cognitive deficits with changes in both cholinergic and GABAergic systems in the frontal cortex. The authors stress that the learning deficit observed in aged rats cannot be explained solely by a reduction in cholinergic receptor sensitivity and that an age-related increase in GABAergic tone may be a more important determinant of cognitive impairment (68).

Concerning neuropeptides, some reports, although controversial, show that TRH, met-enkephalin and somatostatin also play important roles in memory, learning and conciousness. Regional distribution of neuropeptides do not correlate with those of their receptors. Furthermore, age-related changes—usually decreases—occur mainly in receptor but not neuropeptide concentrations. In one study, none of the neuropeptides TRH, met-enkephalin, dynorphin, substance P, or somatostatin, in different brain areas, changed after intraperitoneal injections of 1 mg/kg of dihydroergotoxine for 14 days; the met-enkephalin and TRH receptor levels in the cerebral cortex of aged rats were markedly lower than those of young adult rats. Following the intraperitoneal administration of dihydroergotoxine, these receptor levels showed significant recovery in the cerebral cortex, but not in the other brain sites examined (69).

Low cortical concentrations of somatostain have been reported in elderly people and in those with AD (70, 71). Moreover, the density of somatostatin receptors is also low in the cerebral cortex of AD (70). Lesioning of the

nucleus basalis magnocellularis and administration of cysteamine, a somatostatin depletor, results in severe impairment of memory acquisition in behavioral tasks (72). These results suggest that changes in the brain somatostatinergic transmission are involved in the cognitive deficits in the experimental animal models of aging and dementia presently used. Dihydroergotoxine restores somatostatin receptor binding in the brains of aged rats, but the somatostatin concentration in some brain areas does not change after long-term administration of this drug (73).

These findings suggest that long-term administration of some CNS-acting drugs like dihydroergotoxine or bifemelane has no effect on neuropeptide-containing neurons, but results in specific recovery of age-associated reduction of receptor binding. This, in turn, supports our statement above that the evaluation of receptor number and their binding activity is a necessary complement to neurotransmitter concentration measurements, and that it is indispensable for the proper evaluation of neurotransmitter-mediated neural systems in CNS.

Alzheimer's Disease

Alzheimer's disease (AD) is the most common cause of progressive cognitive failure in aged humans. This disorder is characterized by a progressive neuronal degeneration in the hippocampus, generation of extracellular amyloid plaques in many areas of gray matter, death of neurons with the formation of abnormal cytoskeletal structures and several biochemical abnormalities, including neurotransmitter and receptor defects. Beta amyloid protein (β-A4) plays a dominant role in many current theories of the pathogenesis of this disease (74–77), although it is still not clear if its deposition is the cause or the effect of other processes causing cell death, such as inflammation (78), free oxygen-derived radicals (79, 80) or membrane alterations (altered signal transduction) (81).

A marked loss of presynaptic cholinergic indices in the cerebral cortex and limbic system structures is known to be the main neurochemical event in AD. As mentioned before, the ACh-mediated neuronal system, plays a fundamental role in higher cognitive functions (memory, learning and recognition). Memory is the earliest and most profoundly affected cognitive domain in AD. In patients with ATD, ACh is markedly reduced in the cerebral cortex but the most dramatic abnormality is a marked decrease in CAT activity in the cerebral cortex and hippocampus (11, 82–84). Furthermore, biochemical changes involve deficits in multiple neurotransmitters,

receptors and related enzymes. All the alterations discussed in the precedent section are also present in the brains of ATD patients. In fact, for the most part, the distinction between normal aging and ATD is quantitative rather than qualitative (Fig. 3). Nevertheless, in the case of certain neurotransmitters (e. g. biogenic amines or excitatory amino acids) and their enzymatic markers, the neurochemical evidence is not as robust as in the case of the cholinergic system, due in part to less satisfactory postmortem measurements. The detailed information about these changes, by brain region, is summarized in Table 1.

An evaluation of the cholinergic system must examine not only ACh content and CAT activity, but also ACh receptors and signal transduction through intracellular second messenger systems.

Following the demonstration of significant presynaptic changes, researchers began to look for the presence of alterations in muscarinic receptor levels. In most of these studies, MCR binding, including M_1-R binding, was reported to be normal (38, 85, 86). In other reports, however, MCR binding appeared to be slightly reduced (87, 88). Later studies suggested significant reductions of the M_1-R and high affinity state M_1-R (H M_1-R) subtypes in the frontal cortex of AD but not ATD (89–92). In one of these studies (89), in addition to the macroautoradiographic determination of MCR distribution according to subtype, the authors determined the distributional and quantitative changes in forskolin and phorbol ester binding in the frontal cortex as indicators of the second messenger systems. The use of [^{3}H]QNB, which binds both pre-and post-synaptic MCR and therefore detects total MCR, revealed complete destruction of the laminar structure of cortical MCR not only in AD, but also in ATD brains at autopsy. Together with the observed reduction in CAT activity, the presynaptic ACh neurons were significantly abnormal in AD and ATD frontal cortex, particularly in terms of their distribution. Furthermore, patients with ATD showed higher M_1-R concentrations and this increase is thought to reflect secondary up-regulation due to degeneration of ACh neurons. Also the loss of the laminar structure of M_1-R indicates destruction of post-synaptic structures.

The first step in MCR signal transduction involves G-proteins. Two or more G-proteins bind the MCR and activate several second messenger systems, including those that suppress adenylate activity (93, 94), increase inositol phospholipid turnover (95–97) and activate K^+

channels (98). Because G-proteins mediate coupling between MCR and the second messenger systems, Ogawa *et al.* evaluated the effect of the addition of GTP on agonist-displacement curves in $[^3H]$QNB binding assays, and found that GTP had little effect in the control subjects, with only a twofold increase in the IC_{50}. On the other hand, in the AD patients, the IC_{50} with GTP was thirteen times higher than that without GTP (89). Functional determination requires not only binding studies, but also studies of G-protein and second messenger systems, in both ATD and normal aging, with a variety of approaches, ranging from genetic to behavioral.

The etiology of ATD is controversial and the mechanisms inducing cellular neuropathology underlying it are still not fully understood. AD is a heterogeneous disorder lacking an effective therapy and may require a wide variety of strategies addressing all areas of therapeutic endeavor. The evaluation of drugs for patients with senile cognitive disorders is a very difficult area and therapeutic options, although increasing in number, have not been very successful.

Among the different pharmacological strategies, the cholinergic approach to ATD treatment has received the most attention owing to the biochemical and pharmacological evidence, mentioned above, of the crucial role of ACh in cognitive functions. Several groups have used cholinergic deficit as an endpoint for pharmacological manipulations in the cerebral cortex of ATD patients and drug therapy, especially during the last decade, relied on boosting the cholinergic system either by promoting ACh synthesis with the administration of ACh precursors, like choline and lecithin (99, 100), or by the prevention of ACh catabolism using AChE inhibitiors such as physostigmine and its analogs (101). The latter approach has shown the most promising results. However, the clinical efficacy of this therapy has not been satisfying and none of these approaches eliminate the symptoms of dementia, although some reports showed that treatment with tacrine improved cognitive functions in some patients with ATD (102-104). Tacrine studies are, perhaps, the best documented clinical efficacy trials using AChE inhibitors. Tacrine is a reversible centrally acting inhibitor of AChE activity that indirectly elevates ACh levels in the brain of animals and improves cognitive performance in rodents and monkeys. One of these clinical studies with tacrine claimed remarkable effects in AD patients (104). This study, and a larger multicenter trial showing some efficacy of tacrine in AD (105), prompted the Food and

Drug Administration (FDA) approval of tacrine in the United States for the specific treatment of memory loss in AD patients,

These trials, however, were limited by short durations of treatment and low dosages. Subsequently, a trial with a longer treatment interval (106) demonstrated a dose-effect relationship with higher doses of tacrine associated with greater efficacy. Tacrine exerts a marked therapeutic effect only at doses which are not tolerated by many patients and gastric problems, elevation of liver enzymes and hepatotoxicity have been reported in 25 % of patients taking the drug (107). Only a minority of patients with mild to moderate AD can be treated with tacrine. For this minority, however, tacrine is the most promising treatment option presently available (108).

With the approval of tacrine, similar symptomatic therapies are likely to become available in the near future. In an effort to minimize the side effects, several other AChE inhibitors are under study. An investigational AChE inhibitor, velnacrine maleate, produced modest though significant benefits in a clinical trial with AD patients, but reversible abnomal liver function was also seen in 24 % of the patients with the highest dose of the drug (109), although some authors suggest the hepatotoxicity is less than with tacrine (110).

Another agent, THB013, is more efficacious in inhibiting plasma AChE as well as in blocking scopolamine-induced disruption of spatial learning (111). It is possible that THB013, with more potent cholinergic effects than tacrine, could be also useful for the treatment of AD.

A series of new piperidine derivatives have been found to strongly inhibit AChE. One of them, E2020 (donepezil hydrochloride) is about 15 times more potent than tacrine when tested *in vitro*, is more selective for AChE than tacrine and physostigmine, and penetrates easily into the brain (112). These characteristics point to E2020 as another potential tool for the treatment of ATD.

The efficacy of AChE inhibitors is dependent upon endogenous cholinergic neurotransmission. With disease progression and the loss of presynaptic basal forebrain cholinergic neurons, the efficacy of these drugs may diminish. This explains why research efforts in the cholinergic therapeutic strategy switched to exogenous muscarinic agonists directly acting at these sites. The utility of existing muscarinic agonists like arecholine and oxotremorine is limited and in some studies they were found to be ineffective (99-101). Efforts to design muscarinic agonists with improved pharmacologic profiles

Table I   Alterations of CNS enzymes, neurotransmitters and receptors in Alzheimer's disease

Neurotransmitter/ enzyme/receptor	Cerebrum Frontal cortex	Cerebrum Temporal cortex	Hippocampus	Amygdala	Nucleus basalis of Meynert (substantia innominata)	Caudate nucleus	Putamen	Globus pallidus	Thalamus	Hypothalamus	Substantia nigra	Locus coeruleus	Cerebellum	CSF	Reference number
CAT															11, 38, 49, 63, 82, 84, 92, 146, 147
mACh-R															
M1-R															
M2-R															
nACh-R															
Noradrenaline															144, 148
α1-R															
α2-R															
β1-R															
β2-R															
Dopamine															88, 144, 147, 148
dopa decarboxylase															
HVA															
DBH															
D1-R															
D2-R															
5-HT															51, 144, 147
5-HIAA															
5-HT1-R															
5-HT2-R															
Glutamate															20, 65, 144, 147
glutamate-R															
NMDA-R															
KA-R															
QA-R															
GABA															67, 68, 144, 147
GABA A-R															
GABA B-R															
Benzodiazepine-R															
SS															69, 70, 71
SS-R															
CRH															69, 71, 144
CRH-R															
CCK-8															144
CCK-8-R															
VP															144, 145
OX															

R: receptor, ↓: decrease, =: no change, ^: increase
CAT: choline acetyltransferase HVA: homovanillic acid DBH: dopamine β-hydroxylase 5-HIAA: 5-hydroxyindole acetic acid SS: somatostatin CRH: corticotropin releasing homone CCK-8: cholecystokinin-octapeptide
VP: vasopressin OX: oxytocin

are under way.   Exogenous agonists may substitute ACh itself. Moreover, the discovery of different muscarinic receptor subtypes offers new opportunites to face the problem in a very specific way. CI-979 and PD-142505 are the first attempts of a new class of muscarinic agonists. CI-979 is a non-subtype selective, partial muscarinic agonist that enhances cognitive performance, increases central cholinergic activity in rodents and is well tolerated by normal healthy volunteers at single and multiple doses (113).

BIMC182, a new functionally selective M_1-R agonist, behaves as a full agonist at M_1-R, as partial agonist at M_2-R and M_3-R sites and has been suggested as a potentially useful agent for the treatment of dementia disorders (114). Nevertheless, the potential usefulness of cholinomimetics in treating cognitive decline accompanying AD remains to be clearly established.

The ineffectiveness of the so-called supplementation therapies is basically due to the destruction of the laminar distribution of MCR in the cerebral cortex (89), the reduction of high-affinitiy agonist binding to MCR (89, 115) and the reduction, or disintegration, of second messenger systems (90). Besides, the deficits in AD are not restricted to one region or to neurons releasing only ACh as neurotransmitter. Projection and local circuit neurons releasing many other neurotransmitters are involved including neurons releasing norepinephrine, dopamine, serotonin, glutamate, GABA, somatostatin, oxytocin, cholecystokinin, *etc.* (Table 1). Thus, there is no direct evidence that damage to cholinergic neurons in the basal forebrain is solely responsible for the cognitive dysfunction in AD.

The development of drugs with multiple effects on both pre- and post-synaptic ACh neurons is desirable. Some authors have reported that reduced CAT and MCR concentrations in senescent rat brain returned to normal following long-term administration of dihydroergotoxine or bifemelane (60, 116). Thus, it appears that the senescent brain is able to retain its function and plasticity. Unfortunately, the findings that the MCR laminar structure is disrupted in the frontal cortex of ATD patients suggest that even drugs with post-synaptic actions would be ineffective in advanced cases. There are, at the moment, other possible directions for effective ATD drug therapy and some of them will be described in the following section.

Other Possible Therapeutic Directions

In addition to what has been discussed about the etiological and pharmacological considerations of ATD in the previous section, it is very important to mention some other "logical" therapeutic approaches aimed at reducing the progression of ATD or the consequences of neuronal loss.

Recently, a wide array of treatment strategies have been actively tested and they include different substances. Until now, however, it has been practically impossible to define a coherent approach in the search for the "cognitive enhancers", "nootropics" or "antidementia" drugs. Besides, the treatment must address not only core symptoms but also the secondary psychiatric symptoms which occur during the progression of the disease.

The lack of a suitable animal model has often been pointed out as a major obstacle to researchers in elucidating the pathogenesis and treatment of this disease. Games and colleagues (117), using transgenic mice, had recently described a model that expresses high levels of β-amyloid precursor and develops many other signs characteristic of ATD. The use of transgenic animals mimicking the modifications of ATD pathology will help cosiderably in the future in testing new or existing therapies for the disease, and in the meantime, the following approaches deserve attention.

Reduction of β-amyloid production and/or neurotoxicity.   The first logical strategy is to try to reduce the abnormal β-amyloid production and deposition, for example, by modifying the activity of proteases, or study potential protective effects of substances against β-amyloid fragments or other neurotoxic factors such as free radicals (74).

Preventive treatment.   Because the etiology is controversial and almost totally unknown, primary prevention is impossible. Secondary prevention should focus on stress and dietary factors as overactivity in the hypothalamic-pituitary-adrenal axis (possibly indicating maladaptation to stress) and vitamin B 12 deficiency are common (118). Nerve growth factors (NGF) and gangliosides have been used to inhibit progression of the disorder, but this treatment is still at an experimental stage (119), as are efforts to prevent β-amyloid formation. One possibility is the use of implants of polymer-encapsulated human NGF-secreting cells (120). This encapsulated system may provide therapeutically effective levels of a number of neurotrophic factors, alone or in combination. Nevertheless, levels of the low-affinity receptor for NGF appear to be at least stable in AD basal forebrain, and the finding of AD-related increases in

cortical NGF brings into question whether endogenous NGF levels are related to cholinergic atrophy and whether the provision of NGF will be useful in treating this disease (121). Further studies of the degree and the distribution of NGF and their low-affinity receptors within the human brain in normal aging and ATD are necessary before engaging in clinical trials.

Gangliosides are glycosphingolipids localized in the plasma membrane of vertebrate cells, with higher concentrations found in the mammalian brain. There are four major brain gangliosides, the simplest being the GM-1 ganglioside. Gangliosides have been used in the treatment of peripheral nerve damage and other CNS injuries or diseases. GM-1 has recently been shown to have a significant beneficial effect in AD (122).

Long-term estrogen replacement therapy. A potential role for long-term low-dose estrogen replacement therapy has been suggested recently, since some studies and a few case reports showed improvement in cognitive functions, dementia and daily activities in women with mild moderate ATD (123, 124).

Nootropic drugs. The nootropics are a group of compounds which are meant to have possible cognition-enhancing effects (125), but opinions about these preparations are highly divergent. All efforts to elucidate the mechanism of action of these agents have so far been fruitless. A nootropic compound may be defined as a substance which facilitates integration through activation of physiologic adaptation (126). The possibility is entertained that nootropics might interfere with both the dementing process and the aging process.

Four "classic" nootropics drugs (piracetam, pramiracetam, oxiracetam and aniracetam) have been widely studied but only some trials with piracetam and aniracetam in elderly patients with mild to moderate cognitive impairment due to senile dementia have shown some beneficial results (127, 128).

A different kind of nootropic drug is cerebrolysin, a peptidergic agent with a multimodal mechanism of action. Experimental studies have shown that it has a regulatory effect on energy metabolism, a positive influence on behavior through neuromodulation and a neurotrophic stimulatory effect (129). So it is expected to have a positive influence on neurodegenerative diseases such as ATD.

Another drug that has been recently mentioned as a nootropic is the BDZ antagonist flumazenil. Chronic administration of flumazenil significantly protects rats

from age-related loss of cognitive functions. In the previous section we mentioned a possible explanation for such an effect (BDZ/GABAergic hypothesis of aging). The review of human clinical and animal data indicates that flumazenil has nootropic actions by enhancing vigilance, cognitive and habituation processes (67).

Of course, there are many other drugs belonging to such diverse categories as calcium antagonists (130), angiotensin-converting enzyme inhibitors (131), NMDA blockers, glycine antagonists (132), monoamine oxidase inhibitors (133), corticotropin analogs (134), *etc.*, all capable of improving performance on behavioral tests in animal models, that have also been termed "nootropics" or "memory enhancers", but none of these therapies had shown significant effects on the cognitive decline in AD.

Anti-inflammatory drugs. Numerous markers of inflammation have been reported in the AD brain (78). Inflammation is not simply a response to already existing Alzheimer's pathology, but ultimately becomes a significant source of pathology. Inflammation may be a final common pathogenic pathway through which β-amyloid deposition, neurofibrillary tangle formation and other events are linked to the loss of neurons and their connections. The reported lower prevalence of ATD in subjects treated for rheumatoid arthritis led to speculations that long-term use of anti-inflammatory drugs may be a potential treatment for ATD. A small clinical trial using indomethacin produced evidence of significantly higher neuropsychological tests scores compared to placebo-treated Alzheimer's patients (135). In addition, non-steroidal anti-inflammatory drugs and other anti-inflammatory drugs have a relevant capacity to scavenge free radicals and suppress the generation of reactive oxygen metabolites by phagocytes (136).

Drugs acting on free oxygen radical-mediated mechanisms. Cellular events involving "oxidative stress" may be one of the basic pathways leading to neurodegeneration in ATD. There are indications for an increased activity or impaired defense mechanisms of free oxygen radicals in ATD. The exacerbated oxidative stress, indicated by enhanced activity of glucose-6-phosphate dehydrogenase, is associated with pronounced changes in neuronal lipofuscin deposition (137), excess protein oxidation and accompanying dysfunction of brain enzymes (80). Several potential sources of oxidative stress should be considered in the pathogenesis of ATD (iron concentration, increased protein modification by reducing sugars, microglial activa-

tion, increased levels of aluminum in neurofibrillary tangles, *etc.*) (80).

In addition to oxidative modifications occurring in the brain, the study of peripheral tissues has revealed decreases in the blood plasma levels of the antioxidant vitamins A and E, as well as those of carotenoids in ATD (138).

Since specificity of oxygen radical toxicity has not yet been proven, "partial" neuroprotective drugs might be more beneficial than selective drugs affecting only one pathological mechanism (79). The use of antioxidant vitamin supplements (139), metal ion chelators like deferrioxamine (140) or 21-aminosteroids (141), spin-trap compounds (142), and free-radical scavengers (143) provide valuable examples of potentially effective therapies that deserve thorough investigation.

Concluding Remarks

Much of todays neuropsychopharmacology has been based on the assumption that a biochemical deficit may explain mental and behavioral disturbances and thus be amenable to supplementation biochemical therapy. Reductions in neurotransmitters have indeed been reported in various diseases affecting the CNS, but the replacement of neurotransmitters (or their precursors) has shown therapeutic effects only in Parkinson's disease where the determination of the exact biochemical deficit responsible for the disease and the reproduction of similar biochemical lesions in animal models is feasible. As we have seen, this is not the case with the deficiencies observed in AD or normal aging. Although such lesions occur in cholinergic neurons in the basal forebrain and in their axon terminals in the cortex and hippocampus, they are not confined to these sites. Another reason for the failure of replacement therapy is insufficient receptor function, which prevents neurotransmitters from producing their expected effects. We emphasize that both pre- and post-synaptic markers must be determined to examine the function of CNS and CNS-acting drugs. In addition, we need to study and develop drugs that act directly on receptors, drugs that increase the function or number of receptors and drugs that delay or prevent structural destruction in the different neuronal systems (causal therapy); these drugs have greater therapeutic potential and developing them should be our primary goal. Pharmacological therapy for AD is a major goal of extensive research programs throughout the world. It remains to be demonstrated which of the varied treatment strategies described in the preceding sections have the best future

prospect for reducing the progression or preventing the structural lesions, and modifying the clinical course, of senile neurodegenerative diseases. Other symptomatic therapies are likely to appear in the near future. In the long term, however, prevention and cure will be based on a full understanding of the pathogenesis. In this respect, the current availability of a good animal model (117) would help considerably, both in understanding the relationship of the various aspects of the pathology and in testing new pharmacologically active agents based upon these relationships. Quantitative receptor binding studies, on the other hand, continue to be one of the most important means of exploring the molecular aspects of synaptic transmission and have proven to be of immense practical importance as rapid and simple screening techniques for the biochemical actions of such pharmacological treatments.

Effectively treating age-related brain disorders remains a crucial challenge and novel therapeutic strategies might succeed either by preventing and treating disease-specific mechanisms or by delaying the aging process itself. Therefore, it is important to discuss every new treatment option not only within the particular theoretical framework of each disease, but from a wider framework that includes the normal aging process and the mechanisms of age-related neurodegeneration.

References

1. Hökfelt T, Johansson O, Ljungdable, Å Lundberg JM and Schultzberg M: Peptidergic neurones. Nature (1980) 284, 515–521.
2. Ogawa N and Nukina I: Dopamine receptors in the central nervous system. Jpn J Neuropsychopharmacol (1983) 5, 897–905.
3. Snyder SH and Yamamura HI: Antidepressants and muscarinic acetyl-choline receptors. Arch Gen Psychiatry (1977) 34, 236–239.
4. Ogawa N, Yamawaki Y, Kuroda H, Nukina I, Ota Z, Fujino M and Yanaihara N: Characteristics of thyrotropin releasing hormone (TRH) receptors in rat brain. Peptides (1982) 3, 669–677.
5. Snyder SH and Bennett JP: Neurotransmitter receptors in the brain: Biochemical identification. Ann Rev Physiol (1976) 38, 153–175.
6. Ogawa N, Mizuno S, Nukina I, Tsukamoto S and Mori A: Chronic thyrotropin releasing hormone (TRH) administration on TRH receptors and muscarinic cholinergic receptors in CNS. Brain Res (1983) 263, 348–350.

7. Bartus RT, Dean III RL, Beer B and Lippa AS: The cholinergic hypothesis of geriatric memory dysfunction. Science (1982) 217, 408 –417.

8. Squire LR: Physostigmine: Effects on retention at different time after brief training. Psychon Sci. (1970) 19, 49–50.

9. Drachman DA and Leavitt J: Human memory and the cholinergic system: A relationship to aging. Arch Neurol (1974) 30, 113–121.

10. Bartus RT and Johnson HR: Short term memory in the rhesus monkey: Disruption from the anticholinergic scopolamine. Pharmacol Biochem Behav (1976) 5, 39–46.

11. Davies P and Mahoney AJF: Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet (1976) 2, 1403.

12. Perry EK, Perry LH, Biessed G and Tomlinson BE: Necropsy evidence of central cholinergic deficits in senile dementia. Lancet (1977) 1, 189.

13. Coyle JJ, Price DL and Delong MR: Alzheimer's disease: A disorder of cortical cholinergic innervation. Science (1983) 219, 1184–1190.

14. Eckerman DA, Gordon WA, Edwards JD, McPhail RL and Gage MF: Effects of scopolamine and amphetamine on the radial maze performance in the rat. Pharmacol Biochem Behav (1979) 12, 545–602.

15. Okaichi H and Jarrad LE: Scopolamine impairs performance of a place and cut task in rats. Behav Neurol Biol (1982) 35, 319–325.

16. Deutch JA: The cholinergic synapse and the site of memory. Science (1971) 174, 788–794.

17. Buresova O and Bures J: Radial maze as tool for assessing the effect of drugs on the working memory of rats. Psychopharmacology (1982) 77, 268–271.

18. Stevens R: Scopolamine impairs spatial maze performance in rats. Physiol Behav (1981) 27, 385–386.

19. Watts J, Stevens R and Robinson C: Effects of scopolamine on radial maze performance in rats. Physiol Behav (1981) 26, 845–851.

20. Ogawa N, Mizukawa K, Haba K, Asanuma M and Mori A: Effects of chronic bifemelane hydrochloride administration on receptors for N-methyl-D-aspartate in the aged-rat brain. Neurochem Res (1992) 17, 689–691.

21. Ogawa N, Mizukawa K, Haba K, Asanuma M and Mori A: Chronic bifemelane hydrochloride administration enhances muscarinic cholinergic receptor binding in the senescent rat brain. J Med (1991) 22, 17 –27.

22. Ogawa N, Haba K, Yoshikawa H, Ono T and Mizukawa K: Comparison of the effects of bifemelane hydrochloride, idebenone and indeloxazine hydrochloride on ischemia-induced depletion of brain acetylcholine levels in gerbils. Res Commun Chem Pathol Pharmacol (1988) 61, 285–288.

23. Yamamoto M and Shimizu M: Effects of indeloxazine hydrochloride (YM-08054) on anoxia. Arch Int Pharmacodyn Ther (1987) 286, 272– 281.

24. Tachikawa S, Harada M and Maeno H: Pharmacological and biochemical studies on a new compound, 2-(7-indenyloxymethyl) morpholine hydrochloride (YM-08054-1), and its derivatives with potential antidepressant properties. Arch Int Pharmacodyn Ther (1979) 238, 815–95.

25. Yamamoto M and Shimizu M: Cerebral activating properties of indeloxazine hydrochloride. Neuropharmacology (1987) 26, 761–770.

26. Tobe A, Egawa M and Nagai R: Effect of 4-(o-benzylphenoxyl-N-methyl amine hydrochloride (MCI-20160) on the scopolamine -induced deficit of spontaneous alteration behaviour in rats. Jpn J Pharmacol (1983) 33, 775–786.

27. Tobe A, Yamaguchi T, Nagai R and Egawa M: Effects of bifemelane hydrochloride (MCI-2016) on experimental amsesia (passive avoidance failure) in rodents. Jpn J Pharmacol (1985) 39, 153–161.

28. Higashida A and Ogawa N: Differences in the acquisition process and

the effect of scopolamine on radial maze performance in three strains of rats. Pharmacol Biochem Behav (1987) 27, 483–489.

29. Ogawa N, Haba K, Sora YH, Higasida A, Sato H and Ogawa S: Comparison of the effects of bifemelane hydrochloride and indeloxazine hydrochoride on scopolamine-induced impairment in radial maze performance. Clin Ther (1988) 10, 704–711.

30. Olton DS and Samuelson RJ: Remembrance of places passed: Spatial memory in rats. J Exp Psychol (Anim Behav) (1976) 2, 97–116.

31. Wirsching BA, Beninger RJ, Jhamandas K, Boegman RJ and ElDefrawy SR: Differential effects of scopolamine on working and reference memory of rats in the radial maze. Pharmacol Biochem Behav (1984) 20, 659–662.

32. Jarrard LE, Kant GJ, Meyerhoff JL and Levy A: Behavioral and neurochemical effects of intraventricular AF64A administration in rats. Pharmacol Biochem Behav (1984) 21, 273–280.

33. Walsh TJ, Tilson HA, DeHaven DL, Mailman RB, Fisher A and Hanin I: AF64A, a cholinergic neurotoxin, selectively depletes acetylcholine in hippocampus and cortex, and produces long term passive avoidance and radial maze deficits in the rat. Brain Res (1984) 321, 91–102.

34. Jarrard LE: Selective hippocampal lesions and behavior: Effects of kinic acid lesions on performance of place and cue tasks. Behav Neurosci (1983) 97, 873–889.

35. Fontana DJ, Inouye GT and Johnson RM: Linopirdine (Dup 996) improves performance in several tests of learning by modulation of cholinergic neurotransmission. Pharmacol Biochem Behav (1994) 49, 1075–1082.

36. Dent GW, Rule BL, Tam SW and De Souza EB: Effects of the memory enhancer linopirdine (Dup 996) on cerebral glucose metabolism in naive and hypoxia-exposed rats. Brain Res (1993) 620, 7–15.

37. Aubert I, Rowe W, Meaney MJ, Gauthier S and Quirion R: Cholinergic markers in aged cognitively impaired Long-Evans rats. Neuroscience (1995) 67, 277–292.

38. Aubert I, Araujo DM, Cecyre D, Robitaille Y, Gauthier S and Quirion R: Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. J Neurochem (1992) 58, 529– 541.

39. Quirion R, Wilson A, Rowe W, Aubert I, Richard J, Doods H, Parent A, White N and Meaney MJ: Facilitation of acetylcholine release and cognitive performance by an M(2)-muscarinic receptor antagonist in aged memory-impaired rats. J Neurosci (1995) 15, 1455–1462.

40. Nabeshima T, Nitta A, Fuji K, Kameyama T and Hasegawa T: Oral administration of NGF synthesis stimulators reduced brain NGF content in aged rats and cognitive dysfunction in basal-forebrain-lesioned rats. Gerontology (1994) 40 (Suppl. 2), 46–56.

41. Foreman PJ, Perez- Polo JR, Angelucci L, Ramacci MT and Tagliatela G: Effects of acetyl-L-carnitine treatment and stress exposure on the nerve growth factor receptor (p75NGFR) mRNA level in the central nervous system of aged rats. Prog Neuropsychopharmacol Biol Psychiatry (1995) 19, 117–133.

42. Consolo S, Arnaboldi S, Giorgi S, Russi G and Ladinsky H: 5-HT4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. NeuroReport (1994) 5, 1230–1232.

43. Early B, Burke M, Leonard BE, Gouret CJ and Junien JL: Evidence for an anti-amnesic effect of JO 1784 in the rat: A potent and selective ligand for the sigma receptor. Brain Res (1991) 546, 282–286.

44. Bronzetti E, Caporali MG, Felici L, Niglio T, Scotti de Carolis A and Amenta F: Muscarinic cholinoceptor subtypes in the rat frontoparietal cortex and ipsilateral lesions of the nucleus basalis magnocellularis. Pharmacology (1993) 46, 301–307.

45. Earley B, Burke M and Leonard BE: Behavioural, biochemical and histological effects of trimethyltin (TMT) induced brain damage in the

rat. Neurochem Int (1992) 21, 351–366.

46. Early B, Biegon A and Leonard BE: Quantitative autoradiographic analysis of muscarinic receptors and quantitative histochemistry of acetylcholinesterase in the rat brain after trimethyltin intoxication. Neurochem Int (198) 15, 475–483.

47. Early B, Glennon M, Leonard BE and Junien JL: Effects of JO 1784, a selective sigma ligand, on the autoradiographic localization of M1 and M2 muscarinic receptor subtypes in trimethyltin treated rats. Neurochem Int (1995) 26, 559–570.

48. Decker MW: The effects of aging on hippocampal and cortical projections of the forebrain cholinergic system. Brain Res Rev (1987) 12, 423–438.

49. Smith TD, Gallagher M and Leslie FM: Cholinergic binding sites in rat brain: Analysis by age and cognitive status. Neurobiol Aging (1995) 16, 161–173.

50. Consolo S, Wang JX, Fiorentin F, Vezzani A and Ladinsky H: In vivo and in vitro studies on the regulation of chlinergic neurotransmission in striatum, hippocampus and cortex of aged rats. Brain Res (1986) 374, 212–218.

51. Meek JL, Bertilsson L, Cheney DL, Zsilla G and Costa E: Aging induced changes in acetylcholine and serotonin content of discrete brain nuclei. J Gerontol (1977) 32, 129–131.

52. Tanaka K, Ogawa N, Asanuma M, Kondo Y and Mori A: Chronic administration of acetylcholinesterase inhibitor in the senescent rat brain. Neurobiol Aging (1994) 15, 721–725.

53. Noda Y, McGeer PL and McGeer EG: Lipid peroxides in brain during aging and vitamin E deficiency: Possible relations to changes in neurotransmitter indices. Neurobiol Aging (1982) 3, 173–178.

54. Springer JE, Tayrein MW and Loy R: Regional analysis of age-related changes in the cholinergic system of the hippocampal formation and basal forebrain of the rat. Brain Res (1987) 407, 180–184.

55. Lippa AS, Pelham RW, Beer BC, Critchett DJ, Dean RL and Bartus RT: Brain cholinergic dysfunction and memory in aged rats. Neurobiol Aging (1980) 1, 13–19.

56. Norman AB, Blaker SN, Thal L and Creese I: Effects of aging and cholinergic deafferentiation on putative muscarinic cholinergic receptor subtypes in rat cerebral cortex. Neurosci Lett (1986) 70, 289–294.

57. Leong SF, Lai JCK, Lim L and Clark JB: Energy metabolising enzymes in brain regions of adult and aging rats. J Neurochem (1981) 37, 1548–1556.

58. Markman MH, Ahn HS, Thal LJ, Sahrpless NS, Dvorkin B, Horowitz SG and Rosenfeld M: Evidence for selective loss of brain dopamine- and histamine-stimulated adenylate cyclase activities in rabbits with aging. Brain Res (1980) 192, 177–183.

59. McGeer EG and McGeer PL: Neurotransmitter metabolism in the aging brain.; in Neurobiology of Aging, Terry RD and Gershon S eds, Raven, New York (1976) pp389–403.

60. Ogawa N, Nomura M, Haba k, Asanuma M, Tanaka K, Hori K and Mori A: Effects of dihydroergotoxine on central cholinergic neuronal systems and discrimination learning test in aged rats. Brain Res (1992) 586, 229–234.

61. Ogawa N, Asanuma M, Hirata H, Kondo Y, Kawada Y and Mori A: Cholinergic deficits in aged rat brain are corrected with nicergoline. Arch Gerontol Geriatr (1993) 16, 103–110.

62. Asanuma M, Ogawa N, Kondo Y, Hirata H and Mori A: Effects of repeated administration of rolipram, a cAMP- specific phosphodiesterase inhibitor, on acetylcholinergic indices in the aged rat brain. Arch Gerontol Geriatr (1993)16, 191–198.

63. Schulz DW, Kuchel GA and Zigmond RE: Decline in response to nicotine in aged rat striatum: Correlation with a decrease in a sub-population of nicotinic receptors. J Neurochem (1993) 61, 2225–2232.

64. Arendash GW, Sengstock GJ, Sanberg PR and Kem WR: Improved learning and memory in aged rats with chronic administration of the nicotinic receptor agonist GTS-21. Brain Res (1995) 674, 252–259.

65. Greenamyre JT, Penny JB, D'Amato CJ and Young AB: Dementia of the Alzheimer's type: Changes in hippocampal L- [^{3}H] -glutamate binding. J Neurochem (1987) 48, 543–551.

66. Berger W, Deckert J, Hartmann J, Krotzer C, Kornhuber J, Ransmayr G, Heinsen H, Beckmann H and Riederer P: Memastine inhibits[^{3}H] MK-801 binding to human hippocampal NMDA receptors. NeuroReport (1994) 5, 1237–1240.

67. Marczynski TJ, Artwohl J and Marczynska B: Chronic administration of flumazenil increases life span and protects rats from age-related loss of cognitive functions: A benzodiazepine/GABAergic hypothesis of brain aging. Neurobiol Aging (1994) 15, 69–84.

68. Abdulla FA, Abu-Bakra MA, Calamicini MR, Stephenson JD and Sinden JD: Importance of forebrain cholinergic and GABAergic systems to the age-related deficits in water maze performance of rats. Neurobiol Aging (1995) 16, 41–52.

69. Ogawa N, Hirose Y and Nomura M: Biochemical and functional aspects of neuropeptides and their receptors in aged rat brain.; in Recent Research on Neurotransmitter Receptors, Yoshida H eds, Excerpta Medica, Amsterdam (1986) pp56–68.

70. Beal MF, Mazurek M, Tran V, Chatta G, Bind E and Martin J: Reduced numbers of somatostain receptors in the cerebral cortex in Alzheimer's disease. Science (1985) 29, 289–291.

71. Bouras C, De St Hilaire-Kafi D and Constantinidis J: Neuropeptides in Alzheimer's disease: A review and morphological results. Prog Neuro-Psychopharmacol Biol Psychiatry (1986) 10, 271–286.

72. Matsuoka N, Yamazaki M and Yamaguchi I: Changes in brain somatostain in memory-deficient rats: Comparison with cholinergic markers. Neuroscience (1995) 66, 617–626.

73. Tanaka K, Ogawa N, Asanuma M, Hirata H, Kondo Y, Chou H and Mori A: Effects of chronic codergocrine mesylate administration on the brain somatostatinergic system in aged rats. Arch Gerontol Geriatr (1992) 15, 133–139.

74. Dresse A, Marechal D, Scuvee- Moreau J and Seutin V: Towards a pharmacological approach of Alzheimer's disease based on the molecular biology of the amyloid precursor protein (APP). Life Sci (1994) 55, 2179–2187.

75. Goedert M: Molecular dissection of the neurofibrillary lesions of Alzheimer's diseane. Arzneim Forsch Drug Res (1995) 45, 403–409.

76. Lansbury PT: Consequences of the molecular mechanism of amyloid formation for the understanding of the pathogenesis of Alzheimer's disease and the development of therapeutic strategies. Arzneim Forsch/Drug Res (1995) 45, 432–434.

77. Masters CL and Beyreuther K: Molecular neuropathology of Alzheimer's disease. Arzneim Forsch Drug Res (1995) 45, 410–412.

78. Rogers J: Inflammation as a pathogenic mechanism in Alzheimer's disease. Arzneim Forsch Drug Res (1995) 45, 439–442.

79. Frölich L and Riederer P: Free radical mechanisms in dementia of Alzheimer type and the potential for antioxidative treatment. Arzneim Forsch/Drug Res (1995) 45, 443–446.

80. Smith MA, Sayre LM, Monnier VM and Perry G: Radical AGEing in Alzheimer's disease. Trends Neurosci (1995) 18, 172 176.

81. Roth GS, Joseph JA and Mason RP: Membrane alterations as causes of impaired signal transduction in Alzheimer's disease and aging. Trends Neurosci (1995) 18, 203 206.

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

83. Bowen DM, Smith CB, White P and Davison AN: Neurotransmitter-

related enzymes and indices of hypoxia in senile dementia and other abiotrophies. Brain (1976) **99**, 459 496.

84. Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT and De Lony MR: Alzheimer's disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann Neurol (1981) **10**, 122–126.

85. White P, Hiley CR, Goodhart MJ, Carrasco LH, Keet JP, Williams IEI and Bowen DM: Neocortical cholinergic neurons in elderly people Lancet (1977) **i**, 668–670.

86. Davies P and Verth AH: Regional distribution of muscarinic acetylcholine receptors in normal and Alzheimer's type dementia brains. Brain Res. (1978) **138**, 385–392.

87. Rinne JO, Rinne JK, Laakso K, Paljarvi L and Rinne UK: Reduction in muscarinic receptor binding in limbic areas of Alzheimer brain. J Neurobiol Neurosurg Psychiatry (1984) **47**, 651–652.

88. Reisine TD, Yamamura HI, Bird ED, Spokes E and Enna SJ: Pre and postsynaptic neurochemical alterations in Alzheimer's disease. Brain Res (1987) **159**, 477–481.

89. Ogawa N, Mizukawa K, Asanuma M and Mori A: Abnormalities in muscarinic cholinergic receptors and their G-protein coupling systems in cerebral frontal cortex in Alzheimer's disease. Arch. Gerontol Geriatr (1993) **17**, 77–89.

90. Ogawa N, Mizukawa K, Hada K, Yoshikawa K and Kanazawa I: Alterations in muscarinic cholinergic receptors and the second messenger system in the cerebral cortex in Alzheimer-type dementia; in Basic, Clinical and Therapeutic Aspects of Alzheimer's and Parkinson's Diseases, Nagatsu T, Fisher A and Yoshida M eds, Plenum Press, New York (1990) pp553–558.

91. Griffiths PD, Perry RH and Crossman AR: A detailed anatomical analysis of neurotransimitter receptors in the putamen and caudate in Parkinson's disease and Alzheimer's disease. Neurosci Lett (1994) **169**, 68–72.

92. Pedigo NW: Neurotransmitter receptor plasticity in aging. Life Sci (1994) **55**, 1985–1991.

93. Ehlert FJ: The relationship between muscarinic receptor occupancy and adenylate cyclase inhibition in the rabbit myocardium. Mol Pharmacol (1985) **28**, 410–421.

94. Olianas MC, Onali P, Neff NH and Costa E: Adenylate cyclase activity of synaptic membranes from rat striatum: Inhibition by muscarinic receptor agonists. Mol Pharmacol (1983) **23**, 393 398.

95. Brown E, Kendal DA and Nahorski SR: Inositol phospholipid hydrolysis in rat cerebral cortical slices: I. Receptor characterisation. J Neurochem (1985) **42**, 1379–1387.

96. Gonzalez RA and Crew FT: Cholinergic and adrenergic-stimulated inositide hydrolysis in brain: Interaction, regional distribution, and coupling mechanisms. J Neurochem (1985) **45**, 1076–1084.

97. Jacobson MD, Wusteman M and Downes CP: Muscarinic receptors and hydrolysis of inositol phospholipids in rat cerebral cortex and parotid gland. J Neurochem (1985) **44**, 465 472.

98. Pfaffinger PJ, Martin JM, Hunter DD, Nathanson NM and Hille B: GTP binding proteins couple cardiac muscarinic receptors to a K⁺ channel. Nature (1985) **317**, 536–538.

99. Boyd WD, Graham-White J, Blackwood G, Glen I and Mcqueen J: Clinical effects of choline in Alzheimer senile dementia. Lancet (1977) **ii**, 711.

100. Smith CM, Swash M, Exton-Smith AN, Phillips MJ, Overstall PW, Priper ME and Bailey MR: Choline therapy in Alzheimer's disease. Lancet (1978) **ii**, 318.

101. Muramoto O, Sugishita M, Sugita H and Toyokura Y: Effect of physostigmine on constructional and memory tasks in Alzheimer's disease. Arch Neurol (1979) **36**, 501–503.

102. Summers WK, Majovski LV, Marsh GM, Tachiki K and Kling A: Oral tetrahydroaminoacridine in long term treatment of senile dementia, Alzheimer type. N Engl J Med (1986) **315**, 1241–1245.

103. Pirozzolo FJ, Baski DJ, Swihart AA and Apple SH: Oral tetrahydroaminoacridine in the treatment of senile dementia, Alzheimer type. N Engl J Med (1987) **316**, 1603.

104. Farlow M, Gracon JI, Hershey LA, Lewis KW, Jadowsky CH and Dolan-Ureno J: A controlled trial of tacrine in Alzheimer's disease. JAMA (1992) **268**, 2523 2529.

105. Davis KL, Thal LJ, Gamzu ER, Davis CS, Woolson RF, Gracon SI, Drachman DA, Schneider LS, Whitehouse PJ, Hoover TM, Morris JC, Kawas CH, Knopman DS, Earl NL, Kumar V, Doody RS and The Tacrine Collaborative Study Group: A double-blind placebo-controlled multicenter study of tacrine for Alzheimer's disease. N Engl J Med (1992) **327**, 1253–1259.

106. Knapp MJ, Knopman DS, Soloman PR, Pendlebury WW, Davis CS and Gracon SI: A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer's disease. JAMA (1994) **271**, 985–991.

107. Watkins PB, Zimmerman HJ, Knapp MJ, Gracon SI and Lewis KW: Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. JAMA (1994) **271**, 992–998.

108. Kurz A, Marquard R and Mosch D: Tacrin: Ein Fortschritt in der Behandlung der Alzheimer-Krankheit? Z Gerontol Geriatr (1995) **28**, 163 168.

109. Antuono PG: Effectiveness and safety of velnacrine for the treatment of Alzheimer's disease: A double-blind, placebo-controlled study. Mentane Study Group. Arch. Intern. Med (1995) **155**, 1766–1772.

110. al-Casey S, Brewster D, Viau C and Acosta D: Effect of glutathione depletion and oxidative stress on the *in vitro* cytotoxicity of velnacrine maleate. Toxicol Lett (1995) **76**, 257–265.

111. Adem A, Mohammed AH, Winblad B and Henriksson BE: A novel cholinesterase THB013: Biochemical and behavioural studies. J Neural Transm Parkinson's Dis (1994) **81**, 139 148.

112. Yamanishi Y, Ogura H, Kosasa T, Araki S, Sawa Y and Yamatsu K: Inhibitory action of E2020, a novel acetylcholinesterase inhibitor, on cholinesterase: Comparison with other inhibitors; in Basic, Clinical and Therapeutic Aspects of Alzheimer's and Parkinson's Diseases. Vol 2. Nagatsu T, Fisher A and Yoshida M eds. Plenum Press, New York (1990) pp409 413.

113. Davis RE, Doyle PD, Carrol RT, Emmerling MR and Jaen J: Cholinergic therapies for Alzheimer's disease: Palliative or disease altering? Arzneim Forsch/Drug Res (1995) **45**, 425–431.

114. Cereda E, Brambilla A, Ezhaya A, Gaida W, Mendla K, Monferini E, Pitsikas N, Schiavi GB and Sagrada A: Medicinal chemistry of muscarinic agonists for the treatment of dementia disorders. Eur J Drug Metab Pharmacokinet (1994) **19**, 179–183.

115. Cortes R, Probst A, Tobler HJ and Palacios JM: Muscarinic cholinergic receptor subtypes in the human brain. II. Quantitative autoradiographic studies. Brain Res (1986) **362**, 239–253.

116. Ogawa N, Asanuma M, Mizukawa K, Hirata H, Chou H and Mori A: Post-ischemic administration of bifemelane hydrochloride prohibits ischemia-induced depletion of the muscarinic M1-receptor and its mRNA in gerbil hippocampus. Brain Res (1992) **591**, 171–175.

117. Games D, Adams D, Alessandrini R, Berthelete P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, Guido T, Hagopian S, Johnson-Wood K, Khan K, Lee M, Leibowitz P, Lieberburg I, Little S, Masliah E, McConlogue L, Montoya-Zavala M, Mucke L, Paganini L, Penniman E, Power M, Schenk D, Seubert P, Snyder B, Soriano F, Tan H, Vitale J, Wadsworth S, Wolozin B and Zhao J: Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. Nature (1995) **373**, 523–527.

118. Gottfries CG: Therapy options in Alzheimer's disease. Br J Clin Pract

(1994) **48**, 327–230.

119. Hellweg R: Trophic factors during normal brain aging and after functional damage. J Neural Transm-Gen Soct (1994) **44**, 209–217.
120. Emerich DF, Winn SR, Harper J, Hammang JP, Baetge EE and Kordower JH: Implants of polymer-encapsulated human NGF-secreting cells in the nonhuman primate: Rescue and sprouting of degenerating cholinergic basal forebrain neurons. J Comp Neurol (1994) **349**, 148–164.
121. Scott SA and Crutcher KA: Nerve growth factor and Alzheimer's disease. Rev Neurosci (1994) **5**, 179–211.
122. Svennerholm L: Gangliosides: A new therapeutic agent against stroke and Alzheimer's disease. Life Sci (1994) **55**, 2125–2134.
123. Ohkura T, Isse K, Akazawa K, Hamamoto M, Yaoi Y and Hagino N: Long-term estrogen replacement therapy in female patients with dementia of the Alzheimer type: 7 case reports. Dementia (1995) **6**, 99–107.
124. Simpkins JW, Singh M and Bishop J: The potential role for estrogen replacement therapy in the treatment of the cognitive decline and neurodegeneration associated with Alzheimer's disease. Neurobiol Aging (1994) **15**, S195–197.
125. Mondadori C: In search of the mechanism of action of the nootropics: New insights and potential clinical implications. Life Sci (1994) **55**, 2171–2178.
126. Ban TA: Psychopharmacology and successful cerebral aging. Prog Neuropsychopharmacol Biol Psychiatry (1995) **19**, 1–9.
127. Croisile B, Trillet M, Fondarai J, Laurent B, Mauguière F and Billardon M: Long term and high-dose piracetam treatment of Alzheimer's disease. Neurology (1993) **43**, 301–305.
128. Lee CR and Benfield P: Aniracetam: An overview of its pharmacodynamic and pharmacokinetic properties, and a review of its therapeutic potential in senile cognitive disorders. Drugs Aging (1994) **4**, 257–273.
129. Ruther E, Ritter R, Apecechea M, Freytag S and Windisch M: Efficacy of the peptidergic nootropic drug cerebrolysin in patients with senile dementia of the Alzheimer type (SDAT). Pharmacopsychiatry (1994) **27**, 32–40.
130. Ingram DK, Joseph JA, Spangler EL, Roberts D, Hengemihle J and Fanelli RJ: Chronic nimodipine treatment in aged rats: Analysis of motor and cognitive effects and muscarinic-induced striatal dopamine release. Neurobiol Aging (1994) **15**, 55–61.
131. Sudilovsky A, Cutler NR, Sramek JJ, Wardle T, Veroff AE, Mickelson W, Markowitz J and Repetti S: A pilot clinical trial of the angiotensin-converting enzyme inhibitor ceranapril in Alzheimer's disease. Alzheimer Dis Assoc Disord (1993) **7**, 105–111.
132. Randolph C, Roberts JW, Tierney MC, Bravi D, Mouradian MM and Chase TN: D-cycloserine treatment of Alzheimer disease. Alzheimer Dis Assoc Disord (1994) **8**, 198–205.
133. Cutler NR, Fakouhi D, Smith WT, Hendrie HC, Matsuo F, Sramek JJ and Herting RL: Evaluation of multiple doses of milacemide in the treatment of senile dementia of the Alzheimer Type. J Geriatr Psychiatry Neurol (1993) **6**, 115–119.
134. Heuser I, Heuser-Link M, Gotthardt U, Grasser A and Holsboer F: Behavioral effects of a synthetic corticotropin 4-9 analog in patients with depression and patients with Alzheimer's disease. J Clin Psycho-

pharmacol (1993) **13**, 171–174.

135. Rogers J, Kirby LC, Hempelman SR, Berry DI, McGeer PL, Kazniak A, Zalinski W, Cofild J, Mansukhani L, Willson P and Kogan F: Clinical trial of indomethacinin in Alzheimer's disease. Neurology (1993) **43**, 1609–1611.
136. Halliwell B, Hoult JR and Black DR: Oxidants, inflammation, and antiinflammatory drugs. FASEB Fed Am Soc Exp Biol J (1988) **2**, 2867–2873.
137. Dowson JH, Mountjoy CQ, Cairns MR and Wilton CH.: Changes in intraneuronal lipopigment in Alzheimer's disease. Neurobiol Aging (1992) **13**, 493–500.
138. Zaman Z, Roche S, Fielden P, Frost PG, Niriella DC and Cayley ACD: Plasma concentration of vitamins A and E and carotenoids in Alzheimer's disease. Age Aging (1992) **21**, 91–94.
139. Clausen J, Nielson SA and Kristensen M: Biochemical and clinical effects of an antioxidative supplementation of geriatric patients: A double-blind study. Biol Trace Elem Res (1989) **20**, 135–151.
140. McLachlan DRC, Dalton AJ, Kruck TPA, Bell MY, Smith WL, Kalow W and Andrews DF: Intramuscular desferrioxamine in patients with Alzheimer's disease. Lancet (1991) **337**, 1304–1308.
141. Hall ED: Lazeroids: Efficacy and anti-oxidant mechanism in experimental cerebral ischemia; in Pharmacology of Cerebral Ischemia, Krieglstein J and Oberpichler H eds, Wissenchaftliche Verlagsgesellschaft mbH, Stuttgart (1990) pp345–350.
142. Carney JM, Starke-Reed PE, Oliver CN, Landum RW, Cheng MS, Wu JF and Floyd RA: Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-tert-butyl-a-phenylnitrone. Proc Natl Acad sci USA (1991) **88**, 3633–3636.
143. Hamada H, Hiramatsu M, Edamatsu R and Mori A: Free radical scavenging action of baicalein. Arch Biochem Biophys (1993) **306**, 261–266.
144. Ogawa N: Alzheimer type dementia; in Brain Receptors: New Edition, Ogawa N ed, Sekai Hoken Tsushinsha, Osaka (1989) pp524–533.
145. Rossor MN, Iversen LL, Mountjoy CQ, Roth M, Hawthorn J, Ang VY and Jenkins JS: Arginine vasopressin and choline acetyltransferase in brains of patients with Alzheimer type senile dementia. Lancet (1980) **ii**, 1367.
146. Smith CJ, Perry EK, Perry RH, Candy JM, Johnson M, Bonham JR, Dick DJ, Fairbairn A, Blessed G and Birdsall NJM: Muscarinic cholinergic receptor subtypes in hippocampus in human cognitive disorders. J Neurochem (1988) **50**, 847–856.
147. Zilles KMQ, Schleicher A, Schroeter M, Kramer M and Wittle O: Plasticity and neurotransmitter receptor changes in Alzheimer's disease and experimental cortical infarcts. Arzneim Forsch/Drug Res (1995) **45**, 361–366.
148. Gottfries CG: Alzheimer's disease and senile dementia: Biochemical characteristics and aspects of treatment. Psychopharmacology (1985) **86**, 245–252.