

# Mechanisms of Amyloid $\beta$ Protein-Induced Modification in Ion Transport Systems: Implications for Neurodegenerative Diseases

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## SUMMARY

1. Alzheimer's disease (AD) is a neurodegenerative disorder that affects the cognitive function of the brain. Pathological changes in AD are characterized by the formation of amyloid plaques and neurofibrillary tangles as well as extensive neuronal loss. Abnormal proteolytic processing of amyloid precursor protein (APP) is the central step that leads to formation of amyloid plaque, neurofibrillary tangles, and neuronal loss.

2. The plaques, which accumulate extracellularly in the brain, are composed of aggregates and cause direct neurotoxic effects and/or increase neuronal vulnerability to excitotoxic insults. The aggregates consist of soluble pathologic amyloid beta peptides  $A\beta P[1-42]$  and  $A\beta P[1-43]$  and soluble nonpathologic  $A\beta P[1-40]$ . Both APP and  $A\beta P$  interact with ion transport systems.  $A\beta P$  induces a wide range of effects as the result of activating a cascade of mechanisms.

3. The major mechanisms proposed for  $A\beta P$ -induced cytotoxicity involve the loss of  $Ca^{2+}$  homeostasis and the generation of reactive oxygen species (ROS). The changes in  $Ca^{2+}$  homeostasis could be the result of (1) changes in endogenous ion transport systems, e.g.  $Ca^{2+}$  and  $K^{+}$  channels and  $Na^{+}/K^{+}$ -ATPase, and membrane receptor proteins, such as ligand-driven ion channels and G-protein-driven releases of second messengers, and (2) formation of heterogeneous ion channels.

4. The consequences of changes in  $Ca^{2+}$ -homeostasis-induced generation of ROS are (a) direct modification of intrinsic ion transport systems and their regulatory mechanisms, and (b) indirect effects on ion transport systems via peroxidation of phospholipids in the membrane, inhibition of phosphorylation, and reduction of ATP levels and cytoplasmic pH.

5. We propose that in AD,  $A\beta P$  with its different conformations alters cell regulation by modifying several ion transport systems and also by forming heterogeneous ion channels. The changes in membrane transport systems are proposed as early steps in impairing neuronal function preceding plaque formation. We conclude that these changes damage the membrane by compromising its integrity and increasing its ion permeability. This mechanism of membrane damage is not only central for AD but also may explain other malfunctioned protein-processing-related pathologies.

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**KEY WORDS:** ion channel diseases; protein aggregation; neurodegeneration; beta amyloid; membrane physiology; Alzheimer's disease; neurofibrillary tangles; senile plaques.

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### LIST OF ABBREVIATIONS

A $\beta$ P[1–42]	Amyloid beta protein, residues 1 to 42
A $\beta$ P[1–43]	Amyloid beta protein, residues 1 to 43
AD	Alzheimer's disease
ADNF-9	Alzheimer's disease neurotrophic factor
ADP	Adenosine 5-diphosphate
APP	Amyloid precursor protein
ATPase	Adenosine 5-triphosphatase
bFGF	Basic fibroblast growth factor
C1300	Rat murine C 1300 neuroblastoma cells
CAPP	C-terminal fragment of sAPP
Cp20	GTP-binding protein that is a member of the ADP-ribosylation factor family
DHP-R	Dihydropyridine receptor
DTT	Dithiothreitol
EAAT1,2	Glial specific glutamate transporters
ESC	Evoked postsynaptic currents
GLUT3	Glucose transport protein
GT	Glucose transporter
HNE	4-Hydroxynonenal
IP <sub>3</sub>	D-myo-inositol 1,4,5-triphosphate
K <sub>ATP</sub>	ATP-sensitive K <sup>+</sup> channel
KCa	Ca <sup>2+</sup> -activated K <sup>+</sup> channel
KCO	K <sup>+</sup> channel openers
L-type VSCC	Voltage-sensitive Ca <sup>2+</sup> channel
MAP	Mitogen-activated protein
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAC	N-Acetylcysteine
NGF	Nerve growth factor
NMDA	Non-N-methyl-D-aspartate
NAPP	N-terminal fragment of sAPP
N1E-115	Neuroblastoma cells
N-type VSCC	Voltage-sensitive Ca <sup>2+</sup> channel
PC	Phosphatidylcholine
PC12h	Rat pheochromocytoma cells
PE	Phosphatidylethanolamine
PLC	Phospholipase C
PS	Phosphatidylserine
P/Q-type VSCC	Voltage-sensitive Ca <sup>2+</sup> channel
ROS	Reactive oxygen species
RY-R	Ryanodine receptor
sAPP	Secreted amyloid precursor protein
SNP	Sodium nitroprusside
SR	Sarcoplasmic reticulum

SSC	Spontaneous postsynaptic currents
Tris	Tris(hydroxymethyl)aminomethane
TTX	Tetrodotoxin
VSCC	Voltage-sensitive Ca <sup>2+</sup> channel
$\omega$ -Aga-IVA	Omega-agatoxin IVA
$\omega$ -CgTX-GVIA	Omega-conotoxin GVIA

## INTRODUCTION

In the literature, Alzheimer's disease (AD) is described as a neurodegenerative disorder that significantly impairs the memory and cognitive functions of the brain. In AD, neurons in the hippocampus and basal forebrain—underlying learning and memory functions—are selectively vulnerable and the changes in these regions are characterized by the formation of amyloid plaques and neurofibrillary tangles, as well as extensive neuronal loss. The precise molecular, biochemical, and cellular events and their sequence that result in synaptic dysfunction and neuronal degeneration in the brain in AD patients are far from being established.

There is a large volume of evidence showing that the presence of A $\beta$ P (A $\beta$ P[1–42], A $\beta$ P[1–43], and A $\beta$ P[1–40]) in the brain is linked to AD. In this review we examine possible mechanisms underlying changes in the cell membrane electrical properties in AD and focus on the mechanisms by which A $\beta$ P affects basic membrane functions, i.e. compartmentalization, selective exchange of cellular products, maintenance of ionic gradients, recognition through receptors and excitation transmission. We rely on pharmacological, ion flux, and electrophysiological evidence to deduce amyloid-induced modification in the membrane properties. We have surveyed and summarized current understanding of the properties of (a) amyloid interaction with ion transport systems and (b) amyloid-formed ion channels. In doing so we discuss recent advances regarding the function of the amyloid peptides and proteins, and how these relate to the pathology of AD. Improved understanding of the molecular basis of A $\beta$ P-induced modifications in the electrical properties of neurons could ultimately lead to a better understanding of the causation of these conditions and the design of rational therapy ideally to prevent them and/or halt and reverse AD symptoms. Here, particular attention is devoted to A $\beta$ P-induced changes in endogenous membrane proteins and to A $\beta$ P-formed channels. We show that transport-protein-mediated changes in the membrane electrical properties lead to changes in Ca<sup>2+</sup> homeostasis and production of ROS causing neurodegenerative disorders.

## APP EFFECTS ON ION TRANSPORT SYSTEMS

The APP gene is located on chromosome 21 and presenilin genes 1 and 2 are located on chromosomes 14 and 1, respectively. The apolipoprotein E gene is located on chromosome 19 and  $\alpha$ 2-macroglobulin gene located on chromosome 12 (see Mattson *et al.*, 1998; St. George-Hyslop, 1990; St. George-Hyslop, 2000, and references within). The biogenesis of A $\beta$ P and cellular processing of APP have been

intensely investigated (see also Barrow *et al.*, 1992; Barrow and Zagorski, 1991; Golde *et al.*, 1992; Haass *et al.*, 1992; Haass and Selkoe, 1993). The A $\beta$ P is derived mainly from proteolytic cleavage of the APP, a highly conserved integral membrane protein with a single membrane-spanning polypeptide. The signal for the enhancement in proteolytic cleaving of the APP that results in the excess production of A $\beta$ P and reduction in the secreted APP is not well-known (see Haass and Selkoe, 1993; Murphy *et al.*, 2000). Recently it has been shown that L-type voltage-sensitive Ca<sup>2+</sup> channels (VSCC) antagonists nifedipine and diltiazem increase APP secretion, and thus indicating A $\beta$ P-induced increase in [Ca<sup>2+</sup>]<sub>i</sub> may interfere with the secretory pathway of APP (Mok *et al.*, 2000). In contrast to the accepted role of A $\beta$ P in producing neurotoxic effects, the physiological role of APP is not well-understood. Mattson (1997) noted that APP is a multifunctional protein and widely expressed in the nervous system. A secreted form of  $\beta$ -APP (sAPP alpha) is released from neurons in response to electrical activity and may function in modulation of neuronal functions. Its role in cell regulation is very likely to be mediated via membrane receptors, i.e. by binding to these receptors it may induce changes in the integral membrane proteins involved in signal transduction, e.g. protein phosphorylation, formation of ligand channels and G-protein-driven release of second messengers, such as cAMP, IP, DAG, and cGMP (see Barger and Mattson, 1995). Alternative enzymatic processing pathways of  $\beta$ -APP can liberate A $\beta$ P to form amyloid fibrils that damage neurons and increase their vulnerability to excitotoxicity (see Hardy and Allsop, 1991; Mattson 1997, 1999; Mattson and Pederson, 1998). It is not known whether the abnormal activity of APP is an isolated phenomenon or whether it is associated with a host of other proteins that become abnormal in their processing and may form amyloid deposits. It is known that several other proteins form amyloid deposits (Sipe, 1994). From a therapeutic perspective it would be important to determine the nature of the signal that triggers protein abnormality and formation of amyloid deposits.

Early studies have examined the role of electrical activity as a possible signal inducing APP release. For example, there is evidence to suggest that Na<sup>+</sup> channels may play a role in APP release. Nitsch *et al.* (1993) examined the role of neurotransmitter release in response to neuronal activation in regulation of APP processing in the brain. They found that electrical depolarization caused a rapid increase in the release of both neurotransmitters and amino-terminal APP cleavage products but not products from the APP carboxyl terminus. They also used the Na<sup>+</sup>-channel antagonist tetrodotoxin to inhibit the increased release of the amino terminal of APP to confirm that the action-potential formation mediates the release of large amino-terminal APP derivatives. There is also evidence to suggest that the modulation of neuronal excitability may be a major mechanism by which  $\beta$ -APP regulates developmental and synaptic plasticity in the nervous system (Chen *et al.*, 2000; Furukawa *et al.*, 1996). There are only a limited number of studies that examined the interaction of these APP-cleaved derivatives with cell membranes, and their effects on ion transport mechanisms or even the formation of ion channels. Some of these studies used the patch-clamp technique to examine APP modulation of Ca<sup>2+</sup> transport systems as likely candidates underlying changes in Ca<sup>2+</sup> homeostasis. For example, Boddeke *et al.* (1994) reported that APP fragment H<sub>657</sub> – K<sub>676</sub><sup>+</sup> (1–10  $\mu$ M) did not affect

$\text{Ca}^{2+}$  currents per se, but clearly blocked the  $\text{Ca}^{2+}$  current suppression mediated by both adrenergic alpha 2B- and opioid delta receptors in a concentration-dependent manner. Because of the similar interaction of C-terminal APP with adrenergic alpha 2B- and opioid delta receptors they proposed that these effects occur downstream of the receptor, possibly via the GTP-binding protein. Furukawa *et al.* (1996) used the whole-cell perforated patch and single-channel patch-clamp analysis of hippocampal neurons to demonstrate that secreted APPs suppress action potentials and hyperpolarize neurons by activating high-conductance, charybdotoxin-sensitive  $\text{K}^+$  channels. The effects were mediated by cGMP and protein dephosphorylation mechanisms. In addition, these authors showed that activation of  $\text{K}^+$  channels by sAPPs was mimicked by a cyclic GMP analogue and sodium nitroprusside (SNP) and was blocked by an antagonist of cGMP-dependent kinase and a phosphatase inhibitor. They proposed that membrane hyperpolarization due to activation of  $\text{K}^+$  channels mediates the ability of sAPPs to decrease  $[\text{Ca}^{2+}]_i$ .

In addition to the  $\beta$ -APP-modulated  $\text{Ca}^{2+}$ -signaling pathways, a signaling pathway involving cGMP is activated by sAPP alpha and modulates the activities of  $\text{K}^+$  channels and NMDA (non-*N*-methyl-D-aspartate) receptors (see Mattson *et al.*, 1997). Furthermore, the findings of Masliah *et al.* (1998) point to the possibility that excitotoxic injury associated with an imbalance in the ratio of spliced APP forms might lead to cell death via caspase-3 activation. Mutation studies suggest that APP protects against excitotoxic neuronal injuries by regulating the function of the glial glutamate transporters. Masliah *et al.* (2000) reported that transgenic mice expressing the 695-amino-acid form of the human APP showed a significant decrease in aspartate uptake when compared to nontransgenic controls. This decrease in glutamate transporter activity was found to be associated with decreased protein expression of glial-specific glutamate transporters, EAAT1 and EAAT2, but did not affect mRNA levels. These findings were taken to suggest that expression of mutant forms of APP disturbs astroglial transport of excitatory amino acids at the posttranscriptional level leading, in turn, to increased susceptibility to glutamate toxicity (Masliah *et al.*, 2000).

Phospholipase C (PLC) is another regulatory second messenger that has been invoked. According to Buxbaum *et al.* (1994), various first messengers linked to PLC, including acetylcholine and interleukin 1, regulate the production of both sAPP and  $A\beta$ P. They noted that the activation of PLC may affect APP processing by either of two independent pathways, one involving an increase in protein kinase C and the other an increase in cytoplasmic  $\text{Ca}^{2+}$  levels. However, the functional sites of these second messengers on APP remain to be elucidated. Morimoto *et al.* (1998) reported that a secretory form of sAPP reduced the frequency of spontaneous postsynaptic currents (SSCs), whereas the amplitude of impulse-evoked postsynaptic currents (ESCs) was increased by sAPP. They further elucidated these opposing effects on spontaneous versus evoked release by using the specific domain of APP. They found that the C-terminal fragment of sAPP (CAPP) only reduced SSC frequency and did not affect ESCs. By contrast, the N-terminal fragment of sAPP (NAPP) did not affect SSC frequency but did increase ESC amplitude. These findings were taken to suggest that the reduction of SSC frequency by sAPP was mediated by activation of  $\text{K}^+$  channels through a cGMP-dependent pathway, whereas the increase of ESC amplitude

was mediated by a different pathway involving activation of protein kinase(s). These results suggest the potential role of sAPP as a modulator of synaptic activity by two specific domains. In agreement with such a regulatory role for APP, Mattson *et al.* (1998) proposed that altered APP processing may endanger cholinergic neurons by reducing the levels of a secreted form of APP, modulating neuronal excitability and protecting neurons against excitotoxic, metabolic, and oxidative insults.

## PROTEASES

There is evidence for multiple proteolytic activities influencing the generation of various A $\beta$ P fragments (Murphy *et al.*, 1999). Chan and Mattson (1999) reviewed the role of proteases of the caspase and calpain families, which have been implicated in neurodegenerative processes, as their activation can be triggered by Ca<sup>2+</sup> influx and oxidative stress. Caspases and calpains are cysteine proteases that require proteolytic cleavage for activation. APP is one of the substrates cleaved by caspases. Others include cytoskeletal and associated proteins, kinases, members of the Bcl-2 family of apoptosis-related proteins, presenilins, and DNA-modulating enzymes. Calpain substrates include cytoskeletal and associated proteins, kinases and phosphatases, membrane receptors and transporters, and steroid receptors. In addition to the enzymes that are involved in malfunction and fragmentation of APP, there are further enzymes that are enhanced as a result of APP fragmentation. For example, it is thought that A $\beta$ P-induced increase in [Ca<sup>2+</sup>]<sub>i</sub> can activate Ca<sup>2+</sup>-dependent proteases, which degrade particular cytoskeletal proteins, and lipases, which generate free radicals resulting in membrane damage and possible cell death. Metals like Zn<sup>2+</sup>, Cu<sup>2+</sup>, and Al<sup>3+</sup> can be involved by providing catalytic activity to produce free-radical-attacking apolipoprotein E.

## A $\beta$ P ACTIVITY AND SECOND MESSENGERS

The link between A $\beta$ P activity and AD is well-accepted. However, the significance of A $\beta$ P configurations, (e.g. water-soluble, aggregated, and fibrillar) in its cytotoxicity as AD progresses needs to be clarified. The A $\beta$ P cytotoxic effects produced by such configurations could be mediated by activation of one or more second messenger systems. These include

**Ca<sup>2+</sup>:** According to the Ca<sup>2+</sup> hypothesis of brain aging, modification of [Ca<sup>2+</sup>]<sub>i</sub> is the primary mechanism in the pathology of AD. Different methods have been used to provide evidence for the activation of Ca<sup>2+</sup> transport systems underlying A $\beta$ P-induced cytotoxicity. These include evidence obtained from patch-clamp technique, lipid-bilayer technique, capacitive currents, Ca<sup>2+</sup> uptake, Ca<sup>2+</sup> fura-2 imaging, anti-monoclonal antibodies, pharmacological blockers, and biochemical and structural approaches. However, it is important to note that [Ca<sup>2+</sup>]<sub>i</sub> responses to A $\beta$ P are heterogeneous (Jalonen *et al.*, 1997). Additionally, it is not understood which one is the primary system for A $\beta$ P-induced changes in [Ca<sup>2+</sup>]<sub>i</sub> (see sections Intrinsic Ion Transport Systems and Ion Channel Formation).

**IP<sub>3</sub> and modulation of Ca<sup>2+</sup> levels:** IP<sub>3</sub> (D-myo-inositol 1,4,5-triphosphate) is produced by phospholipase C (PLC) and induces Ca<sup>2+</sup> release from the endoplasmic reticulum store. It is a key enzyme in signal transduction and is activated by G proteins linked to muscarinic cholinergic receptors. Deficits in cholinergic transmission have been linked to AD (Strosznajder *et al.*, 1999). In synaptic plasma membranes, water-soluble AβP[25–35] increased PLC activity and IP<sub>3</sub> production in the absence of Ca<sup>2+</sup>. However, aggregated AβP fragment significantly inhibited PIP<sub>2</sub>-PLC only in the presence of endogenous Ca<sup>2+</sup>. Depending on aggregation state and Ca<sup>2+</sup> concentration, AβP modulates IP<sub>3</sub> production differently and exclusively in brain synaptic plasma membranes. The alteration in activity caused by aggregation may imply that AβP plaque formation and/or channel formation are necessary for the IP<sub>3</sub> inhibition found in AD brain membranes (Strosznajder *et al.*, 1999). Cytoplasmic Ca<sup>2+</sup> and IP<sub>3</sub> in platelets are observed to be enhanced by AβP[25–35] (Ishikawa *et al.*, 1998). A PLC inhibitor, U73122, completely abolished AβP[25–35]-induced Ca<sup>2+</sup> mobilization. This suggests that PLC and IP<sub>3</sub> production induce Ca<sup>2+</sup> mobilization in platelets.

**cGMP:** AβP appears to interfere with the intracellular cascade in the brain, leading to cGMP formation. This involves activation of NMDA, a type of glutamatergic receptor, which induces Ca<sup>2+</sup>-dependent nitric oxide synthase and NO, release of which then activates soluble guanylate cyclase for the synthesis of cGMP. NMDA becomes less active in the aged brain. AβP[25–35] significantly decreased the synthesis rate of cGMP (Chalmoniuk and Strosznajder, 1999). Paris *et al.* (1999) found that AβP[1–40] did not modulate cGMP production via modulation of nitric oxide synthase or soluble guanylyl cyclase. However, a cGMP phosphodiesterase inhibitor, dipyrindamole, did block the vasoconstrictive effects of AβP, suggesting that AβP could affect the activity of this enzyme to increase cGMP digestion. AβP induction of a microglial proinflammatory response is also prevented by dipyrindamole, and compounds that increase cGMP levels prevent AβP-caused inflammation (Paris *et al.*, 1999). However, Hu and el-Fakahany (1993a,b) found that AβP[25–35] stimulated NO and cGMP production in a neuronal clone. In cortical cultures, AβP destabilizes Ca<sup>2+</sup> homeostasis, but direct neurotoxicity is not observed. In hippocampal cultures, treatment with AβP decreases neuron survival, but the mechanism is unknown. Treatment with dibutyryl cAMP, 8-bromo cAMP, dibutyryl cGMP, and 8-bromo cGMP (the 8-bromo-form is membrane permeable), as well as various Ca<sup>2+</sup>-channel inhibitors, failed to alter AβP cytotoxicity in hippocampal cultures (Whitson and Appel, 1995).

**SNP:** Sodium nitroprusside (SNP) is a spontaneous NO generator. It significantly reduced AβP inhibition of MTT reduction in PC12h and C1300 cells. MTT reduction is considered as the earliest quantifiable marker for cytotoxicity, measuring early redox changes within the cell reflecting the integrity of the electron transport chain (Behl *et al.*, 1994). However, another generator of NO and 8-bromo cGMP failed to affect MTT reduction, so the action of SNP may be mediated by the NO-independent pathway (Takenouchi and Munekata, 1998). Potassium ferrocyanide inhibited AβP activity, so it is possible that the ferrocyanide component of SNP may be partially involved in its effects on AβP activity. The cell death caused by the oxidative species, glutamate and H<sub>2</sub>O<sub>2</sub>, could not be attenuated by SNP, so its effects are

not due to direct antioxidative action (Takenouchi and Munekata, 1998). It has also been noted that AD attributed to a diminished capacity of vascular endothelium to generate nitric oxide (NO). It is argued that vascular NO has the potential to enhance the membrane polarization of cerebral neurons by increasing the open probability of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels; this may protect neurons from the excessive  $\text{Ca}^{2+}$  influx potentiated by  $\text{A}\beta\text{Ps}$  (McCarty, 1998).

### OXIDATIVE STRESS, OXYGEN FREE RADICALS, AND ROS

There is experimental evidence to suggest that  $\text{A}\beta\text{P}$  produces reactive oxygen species (ROS) and causes oxidative stress. These  $\text{A}\beta\text{P}$ -generated ROS and oxidative stress (measured by the increase in the levels of lipid peroxidation products, e.g. HNE (4-hydroxynonenal), is thought to play important roles in the pathogenesis of neurodegenerative disease (Coyle and Puttfarcken, 1993). According to this hypothesis, oxidative stress and free radicals induce DNA damage, protein oxidation (ion transport proteins), and lipid peroxidation, which is consistent with the heterogeneous nature of AD and with aging being a major risk factor (see reviews by Christen, 2000; Keller *et al.*, 1997a,b). As far as ion transport mechanisms are concerned, the use of electrophysiological and molecular biology techniques has shed light on ROS-induced impairment of surface and internal membranes that control cellular signaling. Schubert *et al.* (1995) reported that the protein components of human amyloidoses, e.g.  $\text{A}\beta\text{P}$ , amylin, calcitonin, and atrial natriuretic peptide, mediate their toxic effects on clonal and primary cells via a oxidative mechanism, i.e. free radical pathway.

The deleterious effects of ROS and their interaction with various ion transport proteins underlying the transmembrane signal transduction have been detailed elsewhere (see Kourie, 1997, 1998, 1999a, and references within). ROS-affected transport systems include (1) ion channels:  $\text{Ca}^{2+}$  channels including voltage-sensitive L-type  $\text{Ca}^{2+}$  currents, dihydropyridine-receptor (DHP-R) voltage sensor, ryanodine-receptor (RY-R)  $\text{Ca}^{2+}$ -release channels and  $\text{IP}_3$ -receptor  $\text{Ca}^{2+}$ -release channels,  $\text{K}^+$  channels such as  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels, inward and outward  $\text{K}^+$  currents, ATP-sensitive  $\text{K}^+$  channels,  $\text{Na}^+$  channels,  $\text{Cl}^-$  channels; (2) ion pumps: SR and sarcolemmal  $\text{Ca}^{2+}$  pumps,  $\text{Na}^+/\text{K}^+$ -ATPase ( $\text{Na}^+$  pump) and  $\text{H}^+$ -ATPase ( $\text{H}^+$  pump); (3) ion exchangers:  $\text{Na}^+/\text{Ca}^{2+}$  exchanger,  $\text{Na}^+/\text{H}^+$  exchanger; and (4) ion cotransporters: such as  $\text{K}^+-\text{Cl}^-$  cotransport,  $\text{Na}^+-\text{K}^+-\text{Cl}^-$  cotransport and  $\text{P}_i-\text{Na}^+$  cotransport. The mechanism of ROS-induced modifications in ion transport systems involves (1) oxidation of SH groups located on the ion transport proteins, (2) peroxidation of membrane phospholipids, and (3) inhibition of membrane-bound regulatory enzymes and modification of the oxidative phosphorylation and ATP levels. Several studies by Mattson and coworkers (see Guo *et al.*, 2000; Guo and Mattson, 2000; Mattson *et al.*, 1999) suggest that  $\text{A}\beta\text{P}$ -induced accumulation of mitochondrial ROS causes significant reduction in cellular ATP levels, impairing glucose and glutamate transport systems. These effects were blocked by antioxidants. The alterations in the ion transport mechanisms lead to changes in a second messenger system (primarily  $\text{Ca}^{2+}$  homeostasis) that further augment the abnormal electrical activity

and distortion of the signal transduction causing cell dysfunction, which underlies pathological conditions. Several studies using different approaches provide evidence for A $\beta$ P-generated oxygen free radicals, ROS, and oxidative stress in impairing ion transport. However, it is not known why not all of the above transport systems are affected by A $\beta$ P-generated oxygen free radicals, ROS, and oxidative stress.

Antioxidants have been used not only as investigative tools to confirm that A $\beta$ P cytotoxic effects involve the production of ROS and lipid peroxidation, but also as potential therapeutic agents. Several antioxidants have emerged, which act as free radical scavengers, such as Selegelin, Gingko biloba extract EGB 76, the enzymes superoxide dismutase, peroxidase, and catalase, desferrioxamine (an iron chelating agent) antiinflammatory drugs and estrogens as well as the low-molecular-weight reductants, e.g. glutathione, alpha-tocopherol (vitamin E), and ascorbate (reduced vitamin C) (see Behl *et al.*, 1992, 1994). The cytotoxic effects of A $\beta$ P[25–35] and A $\beta$ P[1–40] and generation of H<sub>2</sub>O<sub>2</sub> in cortical cultured astrocytes were prevented by dithiothreitol (DTT), N-acetylcysteine (NAC), and cyclosporine A, but not by catalase or vitamin E (Brera *et al.*, 2000). It has also been shown that the fragment A $\beta$ P[25–35] induces a rapid, concentration-dependent increase in cytosolic free Ca<sup>2+</sup> levels in suspensions of PC12 neuronal cells (Zhou *et al.*, 1996). Pharmacological studies showed that the action of A $\beta$ P[25–35] is not altered by pretreatment with (1) the Ca<sup>2+</sup> channel blockers nifedipine or cobalt; (2) the depleter of intracellular Ca<sup>2+</sup> stores, cyclopiazonic acid; or (3) the PLC inhibitor, neomycin. However, the increase in Ca<sup>2+</sup> is blocked by the antioxidant lazaroid U-83836E and by vitamin E. The neurotoxic action of A $\beta$ P is likewise blocked by U-83836E and vitamin E but not by nifedipine or cobalt. Zhou *et al.* (1996) concluded that the disruption of Ca<sup>2+</sup> homeostasis and the reduction of cell viability produced by A $\beta$ P in PC12 cells are mediated by free-radical-based processes.

It has also been reported that several different neurotrophic factors can prevent death of cortical and hippocampal neurons induced by excitotoxic and oxidative insults in cell culture and in vivo. Guo and Mattson (2000) reported that a 9-amino-acid bioactive fragment of activity-dependent neurotrophic factor (ADNF-9) enhances basal glucose and glutamate transport, and attenuates oxidative impairment of glucose and glutamate transport induced by A $\beta$ P and Fe<sup>2+</sup>, in neocortical synaptosomes. Similarly, they found that basic fibroblast growth factor (bFGF) was also effective in suppressing oxidative impairment of synaptic transporter functions, while nerve growth factor (NGF) was less effective. They proposed that the mechanism of ADNF-9, bFGF, and NGF action involves suppression of oxidative stress and mitochondrial dysfunction induced by A $\beta$ P and Fe<sup>2+</sup> in synaptosomes.

## A $\beta$ P AND ION TRANSPORT SYSTEMS

The emergence of changes in Ca<sup>2+</sup>-homeostasis as a major event underlying A $\beta$ P-induced cytotoxic effects necessitated the search for A $\beta$ P-modulated endogenous ion transport systems contributing directly or indirectly to changes in the cytosolic Ca<sup>2+</sup> levels. In addition, the ability of A $\beta$ P per se to form pathways for Ca<sup>2+</sup> transport has been investigated.

### Intrinsic Ion Transport Systems

In AD, A $\beta$ P may alter ion transport systems and impair neuronal function to produce symptoms by means other than plaque formation. There is also evidence to suggest that A $\beta$ P induces changes in the ion homeostasis before any visible peptide accumulation (Jalonen *et al.*, 1997). The electrophysiological studies have revealed that A $\beta$ P modifies ion transport, e.g. it increases the amplitude of excitatory responses produced by application of NMDA in septal neurons (Carette *et al.*, 1993), enhances NMDA-receptor-mediated synaptic current in the hippocampus (Wu *et al.*, 1995), induces inward current in cortical neurons (Furukawa *et al.*, 1994), increases membrane conductance in neurons from sympathetic ganglia (Simmons and Schneider, 1993), and reduces GABA-induced Cl<sup>-</sup> current in *aplysia* neurons (Sawada and Ichinose, 1996).

### Active Ion Transport Systems

Active ion transport consumes energy (e.g. ATP), acts against concentration gradients, and is often a substrate-specific carrier protein. Since A $\beta$ P-generated ROS cause the inhibition of phosphorylation and/or splitting of ATP involved in regulating ion channels and driving ion pumps, several studies were conducted to examine the function of ATP-dependent transport systems in A $\beta$ P-treated cells. Such studies have revealed that impairment of ATPase-driven ion transport may play a role in the pathogenesis of neuronal injury in AD (Table I). The molecular events that lead to modification of these enzymes remain controversial. This is generally because ion pumps and exchangers are less understood than ion channels, mainly because of the technical limitations of measuring enzyme activity and of ion flux experiments, where ion transport is deduced from net transport due to influx and efflux processes. Recently, the whole-cell voltage-clamp and patch-clamp techniques have been used for the measurement of a macroscopic Na<sup>+</sup>/K<sup>+</sup> pump in isolated ventricular myocytes (Shattock and Matsuura, 1993) and for the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (Coetzee *et al.*, 1994; Goldhaber, 1996). However, there are presently no techniques available for the recording of unitary currents of a single pump or exchanger protein equivalent to the unitary current recordings of a single channel protein.

*Na<sup>+</sup>/K<sup>+</sup>-ATPase (Na<sup>+</sup> Pump).* Na<sup>+</sup>/K<sup>+</sup>-ATPase is a membrane-bound enzyme composed of two subunits: an  $\alpha$  catalytic subunit with an Mr of 90,000–110,000 and a  $\beta$  subunit with an Mr of 40,000–60,000. The Na<sup>+</sup> pump is important for maintaining coronary tone. The pump transports three Na<sup>+</sup> and two K<sup>+</sup> ions per one ATP hydrolyzed against their concentration gradients, generating internal negative charges. Since the early 1990s (Fiori *et al.*, 1994), it has been known that amyloid deposits correlate with a sharp decrease in the activity of the Na<sup>+</sup>/K<sup>+</sup>-ATPase, affecting electrochemical conduction through the axonal membrane.

Some of the studies that examined the relationships between A $\beta$ P and the reduction in Na<sup>+</sup>/K<sup>+</sup>-ATPase activity and the consequences for cell degeneration are shown in Table I. Mark *et al.* (1995) reported that exposure of cultured rat hippocampal neurons to A $\beta$ P[1–40] or A $\beta$ P[25–35] causes a selective reduction in Na<sup>+</sup>/K<sup>+</sup>-ATPase activity that precedes loss of Ca<sup>2+</sup> homeostasis and cell degeneration. Ouabain, a specific inhibitor of Na<sup>+</sup>/K<sup>+</sup>-ATPase, was used to demonstrate

that impairment of this enzyme was sufficient to induce an elevation of  $[Ca^{2+}]_i$  and neuronal injury. The data suggest that the impairment of  $Na^+/K^+$ -ATPase activity is causally involved in the elevation of  $[Ca^{2+}]_i$  and neurotoxicity. They provided evidence to explain the role of ROS in amyloid pathogenesis by finding that the antioxidant free radical scavengers, vitamin E and propyl gallate, significantly attenuated  $A\beta$ P-induced impairment of  $Na^+/K^+$ -ATPase activity, elevation of  $[Ca^{2+}]_i$  and neurotoxicity, suggesting a role for ROS. They also found that the exposure of synaptosomes from postmortem human hippocampus to  $A\beta$ P resulted in a significant and specific reduction in  $Na^+/K^+$ -ATPase and  $Ca^{2+}$ -ATPase activities, without affecting other  $Mg^{2+}$ -dependent ATPase activities or  $Na^+/Ca^{2+}$  exchange.

Further work was carried out using HNE, which is known to conjugate to specific amino acids of proteins and may alter their function. Mark *et al.* (1997a) tested the hypothesis that HNE mediates  $A\beta$ P[25–35]-induced disruption of neuronal ion homeostasis and cell death. It was found that  $A\beta$ P induced large increases in levels of free and protein-bound HNE in cultured hippocampal cells. These researchers found that HNE impaired  $Na^+/K^+$ -ATPase activity and induced an increase of neuronal intracellular free  $Ca^{2+}$  concentration. HNE was found to be neurotoxic, a quality not shared by other aldehydic lipid peroxidation products. In addition, HNE increased neuronal vulnerability to glutamate toxicity, and HNE toxicity was partially attenuated by NMDA receptor antagonists, suggesting an excitotoxic component to HNE neurotoxicity. Of those compounds examined for attenuation of neurotoxicity, glutathione attenuated the neurotoxicities of both  $A\beta$ P and HNE, whereas the antioxidant propyl gallate protected neurons against  $A\beta$ P cytotoxicity but was less effective in protecting against HNE toxicity. Collectively, the data suggest that HNE mediates  $A\beta$ P-induced oxidative damage to neuronal membrane proteins, which, in turn, leads to disruption of ion homeostasis and cell degeneration (Mark *et al.*, 1997b).

Further research sought to examine compounds that may play a role in preventing or modulating the neurodegenerative process. Because basic fibroblast growth factor (bFGF) exhibits trophic activity for many populations of neurons in the brain, and can protect those neurons against excitotoxic, metabolic, and oxidative insults and because plaques in AD brain contain high levels of bFGF, Mark *et al.* (1997a) examined the role of bFGF in the presence of  $A\beta$ P[25–35] in cultured hippocampal neurons. They found that bFGF protected the neurons through the suppression of ROS accumulation and preservation of  $Na^+/K^+$ -ATPase activity.  $Na^+/K^+$ -ATPase activity was significantly reduced following exposure to  $A\beta$ P[25–35] in control cultures, but not in cultures pretreated with bFGF.  $A\beta$ P[25–35] induced lipid peroxidation, accumulation of  $H_2O_2$ , mitochondrial ROS accumulation, and a decrease in mitochondrial transmembrane potential. In the presence of ouabain (the specific inhibitor of  $Na^+/K^+$ -ATPase activity), bFGF failed to protect the neurons from death. In addition, the effect of HNE on neuron death could not be prevented by the pretreatment with bFGF. These results were taken to suggest that the bFGF acts prior to induction of oxidative stress and impairment of ATPases-dependent transport.

Estrogen replacement therapy reduces the risk of developing AD in postmenopausal women. Since synapse loss is one of the defining features of AD, Keller *et al.*

**Table I.** Effects of A $\beta$ P on Na<sup>+</sup>/K<sup>+</sup>-ATPase (Na<sup>+</sup> Pump)

Membrane, and tissue types	A $\beta$ P type and concentration or 0	Methods	A $\beta$ P-induced effects	Mechanism of inhibition	Reference
Primary rat hippocampal and cortical cell cultures	A $\beta$ P[25-35], A $\beta$ P[1-40]	Na <sup>+</sup> /K <sup>+</sup> ATPase activity	Decrease in cell survival (time and concentration dependent)	Zn <sup>2+</sup> (low concentration 0.5 $\mu$ M) protects against A $\beta$ P; Zn <sup>2+</sup> (high concentration 5 $\mu$ M) enhances A $\beta$ P toxicity. Protective effects of Zn <sup>2+</sup> may be due to enhancement of Na <sup>+</sup> pump functions	Lovell <i>et al.</i> (1999)
Rat primary cortical cultures and hippocampal slices	20 $\mu$ M A $\beta$ P[1-40], 50 $\mu$ M A $\beta$ P[25-35], 20 $\mu$ M A $\beta$ P[1-42]	Ouabain-sensitive Na <sup>+</sup> /K <sup>+</sup> -ATPase Activity [ $P_i$ -formed]	Irreversible inhibition of Na <sup>+</sup> /K <sup>+</sup> -ATPase activity. A $\beta$ P[25-35] and A $\beta$ P[1-40] greater effects than A $\beta$ P[1-42]. In primary cortical neurons, A $\beta$ P[25-35] inhibited Na <sup>+</sup> /K <sup>+</sup> -ATPase prior to cell death. In hippocampal slice, A $\beta$ P[1-40] inhibited Na <sup>+</sup> /K <sup>+</sup> -ATPase after 1h and at nanomolar concentrations		Bores <i>et al.</i> (1998)
Rat cortical synaptosomes	A $\beta$ P[25-35]	Ouabain-sensitive Na <sup>+</sup> /K <sup>+</sup> -ATPase activity assay. Glucose and glutamate transport assays (Radioactive [ <sup>3</sup> H]-2-deoxy-glucose and [ <sup>3</sup> H]-glutamate)	Impairment of Na <sup>+</sup> /K <sup>+</sup> -ATPase activity significantly reduced by pretreatment with 17 $\beta$ -estradiol (estriol) before exposure to FeSO <sub>4</sub> and A $\beta$ P. FeSO <sub>4</sub> and A $\beta$ P reduced glutamine transport (completely prevented by addition of estriol). Progesterone also reduced the effects of FeSO <sub>4</sub> on glucose transport	Estrogens by suppressing membrane lipid peroxidation preserve function of ion-motive ATPases and glutamate and glutamine transporters	Keller <i>et al.</i> (1997a)
Rat hippocampal cell cultures	2-hh exposure to 50 $\mu$ M A $\beta$ P[25-35]	Ouabain-sensitive Na <sup>+</sup> /K <sup>+</sup> -ATPase activity	A $\beta$ P and HNE impaired the function of Na <sup>+</sup> /K <sup>+</sup> -ATPase; induced a concentration-dependent increase of neural [Ca <sup>2+</sup> ] <sub>i</sub> . Concentration- and time-dependent decreases in Na <sup>+</sup> /K <sup>+</sup> -ATPase activity (almost no activity at >10 $\mu$ M). Decrease in neural survival after HNE exposure (exacerbated by Buthionine sulfoximine and glutamate)	HNE toxicity was partially attenuated by NMDA receptor antagonists. [Ca <sup>2+</sup> ] <sub>i</sub> elevation after exposure to HNE prevented by removal of extracellular Ca <sup>2+</sup> . Neural death, only partially protected by propyl gallate, reduced significantly by pretreatment using glutathione ethyl ester	Mark <i>et al.</i> (1997b)

Rat hippocampal cell cultures	20 $\mu$ M A $\beta$ P[25–35]	Ouabain-sensitive Na <sup>+</sup> /K <sup>+</sup> -ATPase activity assay	Induced membrane lipid peroxidation. Na <sup>+</sup> /K <sup>+</sup> -ATPase decreased in cultures exposed to A $\beta$ P for 4 h. bFGF ineffective in protecting against A $\beta$ P-induced impairment of Na <sup>+</sup> /K <sup>+</sup> -ATPase activity when added 6 h prior to, or at the time of exposure to A $\beta$ P	Short pretreatment with bFGF ineffective. Sixteen hours bFGF pretreatment suppressed membrane lipid oxidation and accumulation of ROS. bFGF pretreatment not a protection against HNE toxicity	Mark <i>et al.</i> (1997a)
Rat hippocampal astrocyte cultures	100 $\mu$ M A $\beta$ P[25–35]	Ouabain-sensitive Na <sup>+</sup> /K <sup>+</sup> -ATPase activity assay	Reduced glutamine uptake. Although Na <sup>+</sup> /K <sup>+</sup> -ATPase activity decreased it did not inhibit glutamine uptake. A $\beta$ P produces radicals and ROS, oxidizing glutamine transporter, leading to overactivation of NMDA receptors on adjacent neurons. Intracellular Ca <sup>2+</sup> and ROS not significantly increased	Complete protection provided by PDC and Trolox; DTT however did not	Harris <i>et al.</i> (1996)
Cultured rat hippocampal neurons	20 $\mu$ M A $\beta$ P[1–40], 50 $\mu$ M A $\beta$ P[25–35]	Ouabain-sensitive Na <sup>+</sup> /K <sup>+</sup> -ATPase activity assay	Progressive reduction in neural survival. Rapid, concentration-dependent reduction in Na <sup>+</sup> /K <sup>+</sup> -ATPase activity [Ca <sup>2+</sup> ] <sub>i</sub> . No significant effect on ouabain-insensitive Mg <sup>2+</sup> -ATPase activity in cultures exposed to 50 $\mu$ M A $\beta$ P[25–25]. Ouabain [1–10 $\mu$ M] inhibited Na <sup>+</sup> /K <sup>+</sup> -ATPase activity increased [Ca <sup>2+</sup> ] <sub>i</sub> , causing neural degeneration. Reduced Ca <sup>2+</sup> -ATPase activity (no effect on other Mg <sup>2+</sup> -dependent ATPase activities or Na <sup>+</sup> /Ca <sup>2+</sup> exchange)	Reduction in neural survival minimized in cultures treated with tetrodotoxin and Na <sup>+</sup> -deficient medium. Reduction in Na <sup>+</sup> /K <sup>+</sup> -ATPase activity attenuated in cultures pretreated with vitamin E and propyl gallate (suggests a role for ROS). Tetrodotoxin and Na <sup>+</sup> -deficient medium reduced [Ca <sup>2+</sup> ] <sub>i</sub> elevation	Mark <i>et al.</i> (1995)

(1997a,b) sought to test the hypothesis that estrogens act directly on synapses to suppress oxidative impairment of membrane transport systems. They found that exposure of rat cortical synaptosomes to A $\beta$ P[25–35] and FeSO<sub>4</sub> induced membrane lipid peroxidation and impaired the function of the plasma membrane Na<sup>+</sup>/K<sup>+</sup>-ATPase, glutamate transporter, and glucose transporter. Pretreating the synaptosomes with 17 $\beta$ -estradiol or estriol largely prevented impairment of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, glutamate transport, and glucose transport; other steroids were relatively ineffective. However, the addition of HNE impaired the membrane transport system, despite the presence of 17 $\beta$ -estradiol. This suggests it is the antioxidant property of 17 $\beta$ -estradiol that protects the cells.

Recently, Chauhan *et al.* (1997) examined the expression of Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$  1- and  $\alpha$  3-mRNAs analyzed by in situ hybridization in the superior frontal cortex and cerebellum of brains from AD, nondemented age-matched, and young control subjects. The findings suggest that (a) the increase in  $\alpha$  1-mRNA in AD may be related to an increased reactive gliosis, (b) the decline in  $\alpha$  3-mRNA per individual neuron found in normal aging occur prior to the formation of diffuse plaques and are greatly accelerated in AD, and (c) the decline in  $\alpha$  3-mRNA per neuron found in normal aging may predispose to or potentiate AD pathogenesis.

Lovell *et al.* (1999) examined the effect of Zn<sup>2+</sup> on Na<sup>+</sup>/K<sup>+</sup>-ATPase activity. It has been reported that the protective effect of Zn<sup>2+</sup> against A $\beta$ P cytotoxicity is due in part to the enhancement that prevents the disruption of Ca<sup>2+</sup> homeostasis and cell death associated with A $\beta$ P cytotoxicity in cultured rat cortical cells. However, it has been shown that Zn<sup>2+</sup> at high concentrations accelerates aggregation of A $\beta$ P, the major component of senile plaques in AD. The researchers proposed that Zn<sup>2+</sup> provides protection against A $\beta$ P at low concentrations and enhances cytotoxicity at high concentrations. These effects are mediated via modification of activity.

It is also known that another amyloid, amylin, stimulates glycogenolysis, glycolysis, and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in skeletal muscle. However, it is not known whether amylin stimulates glycolytic ATP production that is specifically coupled to ATP consumption by the Na<sup>+</sup>/K<sup>+</sup> pump. A recent study shows that stimulation of glycolysis and glycogenolysis in resting skeletal muscle by epinephrine or amylin is closely linked to stimulation of active Na<sup>+</sup>/K<sup>+</sup> transport (James *et al.*, 1999). On the other hand, Pittner *et al.* (1995) reported that amylin, unlike A $\beta$ P (see below), does not specifically inhibit glucose transporters in skeletal muscle.

In summary, these findings suggest that one of the effects of A $\beta$ P is to inhibit the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase leading to loss of Ca<sup>2+</sup> homeostasis and cell degeneration. The mechanism by which A $\beta$ P or amylin affects the reaction steps in the function of the pump need further elucidation, e.g. how A $\beta$ P affects the hydrolytic and transport reactions of the pump.

*Mg<sup>2+</sup>-Ca<sup>2+</sup>-ATPase (Ca<sup>2+</sup> Pump).* In contrast to the work on the effects of APP fragments on Na<sup>+</sup>-K<sup>+</sup>-ATPase, little work has been done on the effects of the proteolytic fragments of APP on Mg<sup>2+</sup>/Ca<sup>2+</sup>-ATPase. Kim *et al.* (1998) examined the mechanism of neurotoxicity associated with AD due to proteolytic fragments of APP. They found that carboxy terminal 105-amino-acid (CT105) fragment of APP is a potent inhibitor of Mg<sup>2+</sup>-Ca<sup>2+</sup>-ATPase of endoplasmic reticulum. In contrast A $\beta$ P had no effects on the activity of Mg<sup>2+</sup>-Ca<sup>2+</sup>-ATPase. They suggested that CT105

inhibits the ability of brain microsomes to sequester  $\text{Ca}^{2+}$  and proposed that this mechanism contributes to disruption of  $\text{Ca}^{2+}$  homeostasis involved in inducing the neural toxicity characteristic of AD.

### *Ion Exchanger*

*Na<sup>+</sup>/Ca<sup>2+</sup> Exchanger.* The  $\text{Na}^+/\text{Ca}^{2+}$  exchanger couples the transport of three  $\text{Na}^+$  ions to that of a single  $\text{Ca}^{2+}$  ion in the opposite direction in two consecutive, yet separate, steps (see Condrescu *et al.*, 1997). The  $\text{Na}^+/\text{Ca}^{2+}$  exchanger, together with  $\text{Ca}^{2+}$ -ATPase of the ER/SR, regulates  $\text{Ca}^{2+}$  levels that underlie muscle contractility behavior under both normal and ischemic conditions (see Bourdillon and Wilson, 1981). Little is known of the effects of  $\text{A}\beta\text{P}$  on this exchanger. Kim *et al.* (1999a) investigated the effects of CT105 on  $\text{Na}^+/\text{Ca}^{2+}$  exchanger activity in SK-N-SH human neuroblastoma, in the presence of ouabain and monensin, which are considered to drive the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger in the reverse mode. They found that CT105 induced 50% inhibition in the activity of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger. As to possible ROS effects on this exchanger, the literature reveals that the conflicting effects of ROS on the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger may be partially due to the use of different ROS-generating systems and different parameters to deduce the exchanger activity (Kourie, 1998).

### *Transporters*

Glutamate transporters are important excitatory neurotransmitters in the mammalian central nervous system. The termination of the glutamatergic transmission and the clearance of the excessive neurotoxic concentrations of glutamate are achieved by a high affinity glutamate uptake mechanism.

*Na<sup>+</sup>-Dependent Glutamate Transport.* Another transport system that has been implicated in the pathogenesis of AD is the  $\text{Na}^+$ -dependent glutamate uptake. In addition to the study of APP effects on  $\text{Na}^+$ -dependent glutamate uptake presented above,  $\text{A}\beta\text{P}$  effects were also examined. However, the findings appear contradictory (see Gegelashvili and Schousboe, 1997). On the one hand, the finding that decreased glutamate transporter activity in AD was associated with increased excitotoxicity and neurodegeneration (Masliah *et al.*, 1996) was used to raise the possibility that abnormal functioning of glutamate transport might be involved in the pathogenesis of synaptic damage. It has been reported that treatment of neuronal/astrocytic cultures with  $\text{A}\beta\text{P}$  increases the vulnerability of neurons to glutamate-induced cell death by inhibiting the astrocyte glutamate transporter. This inhibition appears to involve free radical damage to the transporter/astrocytes. Moreover, it was shown that  $\text{A}\beta\text{P}[25-35]$  also inhibited L-glutamate uptake in rat hippocampal astrocyte cultures and this inhibition was prevented by the antioxidant Trolox (Harris *et al.*, 1996). In these studies it is not clear which of the several glutamate transporter subtypes is the most affected in AD and to what extent alteration in the function of this subtype is associated with abnormal expression of APP. Li *et al.* (1997) examined the sensitivity of these subtypes in AD. They found that in astroglia, the subtype EAAT2 is affected in AD and abnormal functioning and/or processing of APP might play an important role in this process.

On the other hand, Noda *et al.* (1999) reported that A $\beta$ P enhanced glutamate release (an outward current, resulting from coextrusion of glutamate and Na<sup>+</sup>) from primary cultured rat microglia via the Na<sup>+</sup>-dependent glutamate transporter that was activated by extracellular K<sup>+</sup>. Similarly, the D,L-threo- $\beta$ -hydroxyaspartate-sensitive inward current, reflecting forward glutamate transport, was also activated by external glutamate. These outward and inward currents, i.e. reverse and forward glutamate transport currents were threefold greater in microglia incubated with A $\beta$ P (5  $\mu$ M) for 4 days. They proposed that the activation of microglia by A $\beta$ P causes an increase in extracellular glutamate concentration via the reverse glutamate transporter, and therefore they suggested that this mechanism might contribute to the pathogenesis of neuronal dysfunction and death in AD.

The above studies suggest that Alzheimer's A $\beta$ P-potentiated glutamate toxicity in neurons is mediated by a mechanism that involves glutamate transporters. However, the factors and molecular mechanisms involved in their aberrant functioning in AD and neurodegenerative diseases need further elucidation.

*Glucose Transport.* In the early 1990s it was shown that amylin at 1  $\mu$ M levels in an isolated rat diaphragm preparation inhibited insulin-stimulated 2-deoxy[<sup>3</sup>H] glucose transport. However, no effect was measured on the basal level of 2-deoxy[<sup>3</sup>H] glucose transport (Hothersall *et al.*, 1990). Similarly, in vitro incubation human muscle strips with human islet amyloid polypeptide causes the inhibition of insulin- and phorbol-ester-stimulated glucose transport (Zierath *et al.*, 1992). Kreutter *et al.* (1993) demonstrated that the inhibition of insulin-stimulated glucose transport by amylin is independent of cAMP and they proposed that it may be mediated by a unique receptor that is distinct from the adenylyl-cyclase-coupled CGRP receptor. This is in contrast to the finding that in isolated rat soleus muscle amylin has no direct effect on glucose transport (Pittner *et al.*, 1995). The findings that amylin induces a large increase in G-6-P, but not in uridine diphosphate-*N*-acetylhexosamines, suggests that the proposed glucosamine system may not be involved in amylin-induced glucose transport inhibition. On the other hand, amylin-induced decreases in uridine diphosphate hexoses suggest uridine or hexosamine-based metabolites may be involved in amylin action (Castle *et al.*, 1998).

A $\beta$ P also has been reported to impair glucose transport. Mark *et al.* (1997c) proposed that A $\beta$ P impairs glucose transport in cultured rat hippocampal and cortical neurons by a mechanism involving membrane lipid peroxidation. They found that A $\beta$ P impaired <sup>3</sup>H-deoxy-glucose transport in a concentration-dependent manner and with a time course preceding neurodegeneration. Other steps in this mechanism that follow the decrease in glucose transport include a decrease in cellular ATP levels and subsequently cell death. They implicated membrane lipid peroxidation by finding that A $\beta$ P-induced decreases in glucose and ATP levels were prevented in cultures pretreated with antioxidants. In contrast, exposure to FeSO<sub>4</sub>, an established inducer of lipid peroxidation, impaired glucose transport. They confirmed membrane peroxidation by immunoprecipitation and Western blot analyses, which showed that exposure of cultures to A $\beta$ P-induced conjugation of HNE to the neuronal glucose transport protein GLUT3. Furthermore, they found that HNE induced a concentration-dependent impairment of glucose transport and subsequent ATP

depletion. Blanc *et al.* (1997) reported A $\beta$ P impairs glucose transport in vascular endothelial cells. They further found that damage and death of ECs induced by A $\beta$ P[25–35] were attenuated by antioxidants, a Ca<sup>2+</sup> channel blocker, and a chelator of intracellular Ca<sup>2+</sup>, and thus proposed the involvement of free radicals and dysregulation of Ca<sup>2+</sup> homeostasis.

### *Ion Channels*

The number of studies on A $\beta$ P effects on Ca<sup>2+</sup> and K<sup>+</sup> channels are on the increase while Na<sup>+</sup> and Cl<sup>-</sup> channels are studied to a lesser extent. Because the effects of modification in these channels were examined in cells treated with A $\beta$ P for hours, there is yet to emerge a clear role of a specific ion channel as a first or as an early target for A $\beta$ P action. Also, the mechanism(s) by which A $\beta$ P affects any of these channels have not yet been elucidated.

*Ca<sup>2+</sup> Channels.* Ca<sup>2+</sup> channels in neurons play an important role in excitation. There are several types of Ca<sup>2+</sup> channels in neurons. Table II summarizes examined Ca<sup>2+</sup> channel types in A $\beta$ P-treated cells. It is apparent that the L-type VSCC is one of the most examined Ca<sup>2+</sup> channels as a possible transport system for A $\beta$ P-induced changes in Ca<sup>2+</sup> homeostasis. In a detailed study Ueda *et al.* (1997) examined the role of VSCC in A $\beta$ P[25–35] neurotoxicity in rat cultured cortical and hippocampal neurons. They suggested that A $\beta$ P[25–35] generates free radicals, which in turn, increase Ca<sup>2+</sup> influx via the L-type VSCC, thereby inducing neurotoxicity. They based this suggestion on the following findings: (a) They noted that when L-type VSCCs were blocked by application of nimodipine, A $\beta$ P[25–35] neurotoxicity was attenuated, whereas the application of omega-conotoxin GVIA (omega-CgTX-GVIA) or omega-agatoxin IVA (omega-Aga-IVA), the blocker for N- or P/Q-type VSCCs, had no effect; (b) They also found that the Ca<sup>2+</sup> current density of A $\beta$ P[25–35]-treated neurons is about twofold higher than that of control neurons and also that A $\beta$ P[25–35] increased Ca<sup>2+</sup> uptake, which was sensitive to nimodipine; (c) They used the 2', 7'-dichlorofluorescein diacetate assay that confirm the ability of A $\beta$ P[25–35] to produce ROS. Nimodipine had no effect on the level of free radicals. In contrast, vitamin E, a free radical scavenger, reduced the level of free radicals, neurotoxicity, and Ca<sup>2+</sup> uptake.

Davidson *et al.* (1994) used the patch-clamp recording techniques to study the effects of A $\beta$ P on cation currents in differentiated mouse N1E-115 neuroblastoma cells. They found that incubation of cells with A $\beta$ P for 24 h increased the median peak of the whole-cell inward current, and shifted the voltage at peak current and that of current activation toward more positive potentials. On the other hand, incubation with the reverse sequence A $\beta$ P (40–1) or A $\beta$ P[25–35] did not produce significant changes in the amplitude or kinetic behavior of the inward current. At the single channel level, A $\beta$ P increased the open probability of cation-conducting ion channels. However, no detailed analysis was conducted to ascertain whether the increase in ion channel activity was the result of activation of additional intrinsic ion channels rather than new channel formation by A $\beta$ P. In addition, the data were from two membrane patches, which may have contained different numbers of active channels.

Table II. Effects of A $\beta$ P and APP Fragment (CT105) on Ca<sup>2+</sup> Transport Systems

Preparation membrane, and tissue types	A $\beta$ P type and concentration	Ca <sup>2+</sup> transport system	Methods	A $\beta$ P-induced effects	Mechanism of action	Reference
Cultured rat hippocampal neurons	1 $\mu$ M A $\beta$ P incubated for 1–4 days; 1 nM, 30 nM sAPP751 incubated for 24 h	Voltage sensitive Ca <sup>2+</sup> channel (VSSC)	Patch-clamp technique (whole-cell)	Decrease in Ca <sup>2+</sup> channel response. sAPPs acts as Ca <sup>2+</sup> blocker to limit number of VSSCs available to respond to A $\beta$ P		Li <i>et al.</i> (2000)
Cultured rat cortical neurons	1 $\mu$ M A $\beta$ P[1–40] incubated for 1 h at 30°C	L- and N-type VSSC	Patch-clamp technique (whole-cell)	Increased Ca <sup>2+</sup> influx and elevated [Ca <sup>2+</sup> ] <sub>i</sub> via activation of L- and N-type VSSC. Increased amplitude of N- and P-type VSSC currents	Effects of A $\beta$ P[1–40] on Ca <sup>2+</sup> blocked by interleukin-1B	MacManus <i>et al.</i> (2000)
Cultured rat cerebellar granule neurons	1 $\mu$ M A $\beta$ P[1–40] incubated for 24 h	N-type VSSC	Patch-clamp technique (whole-cell)	Increase in Ca <sup>2+</sup> channel current at potential positive to 0 mV, with 5-mV shift in channel activation in positive direction and increase in channel deactivation rate	1 $\mu$ M $\omega$ -conotoxin GVIA abolished current increase and increase in rate of channel deactivation	Price <i>et al.</i> (1998)
Differentiated mouse NIE-115 neuroblastoma cells	23 $\mu$ M A $\beta$ P[1–40], A $\beta$ P[25–35], A $\beta$ P[40–1]	L-type VSSC	Patch-clamp technique (whole-cell)	Increase in amplitude of a voltage-gated current (1.8 pA, compared with 0.4 and 0.8 pA in controls). Greater channel activity ( $P_{open} = 0.24$ , $P_{open}$ controls < 0.01). A $\beta$ P[25–35] more toxic than A $\beta$ P[1–40]. No changes in current or cytotoxic effects with A $\beta$ P[40–1]		Davidson <i>et al.</i> (1994)
SK–N–SH cells originated from human neuroblastoma	10 $\mu$ M CT105 fragment of APP	Na <sup>+</sup> –Ca <sup>2+</sup> -exchanger driven in reverse by monensin, an Na <sup>+</sup> ionophore	<sup>45</sup> Ca <sup>2+</sup> uptake in the presence of ouabain, an inhibitor of Na <sup>+</sup> /K <sup>+</sup> -ATPase	CT105-induced inhibition of <sup>45</sup> Ca <sup>2+</sup> uptake was apparent after 1 min	CT105-induced inhibition of Ca <sup>2+</sup> uptake. Vitamine E did not prevent the inhibition	Kim <i>et al.</i> (1999)
Rat brain microsomes	10 $\mu$ M CT105 fragment of APP; 50 $\mu$ M A $\beta$ P[25–35]	Mg <sup>2+</sup> –Ca <sup>2+</sup> -ATPase	<sup>45</sup> Ca <sup>2+</sup> uptake	Pretreatment of the microsomes with CT105 for 3 min inhibited <sup>45</sup> Ca <sup>2+</sup> uptake	CT105-induced inhibition of Mg <sup>2+</sup> –Ca <sup>2+</sup> -ATPase. A $\beta$ P[25–35] is not effective	Kim <i>et al.</i> (1998)

The effects of A $\beta$ P on VSCCs were also measured in cultured rat cerebellar granule neurons using the whole-cell patch-clamp technique (Price *et al.*, 1998). It was found that inhibition of L-type channels with 2  $\mu$ M nifedipine did not prevent the A $\beta$ P-induced rise in Ca<sup>2+</sup> channel current or effects on current activation and deactivation. The N-type Ca<sup>2+</sup> channel antagonist omega-conotoxin GVIA (1  $\mu$ M) abolished the A $\beta$ P-induced current increase and the increase in rate of channel deactivation, but did not prevent the A $\beta$ P-induced shift in the current activation curve. They concluded that A $\beta$ P might exert its effects on cell survival by increasing Ca<sup>2+</sup> influx through N-type Ca<sup>2+</sup> channels in central neurons. A $\beta$ P-mediated VSCC current enhancement is suppressed by secreted APP (Li *et al.*, 2000). Weiss *et al.* (1994) found that the Ca<sup>2+</sup> channel blocker nimodipine attenuated A $\beta$ P-induced neuronal injury, suggesting that Ca<sup>2+</sup> entry through an L-type VSCC may be involved in cell injury. According to Whitson and Appel (1995), however, A $\beta$ P cytotoxicity is unlikely to be mediated by direct interaction with the N- or L-channels. They reported that antagonists to N-channels, L-channels, NMDA-receptor channels, and exogenous cyclic nucleotides had no effect on the survival of hippocampal neurons exposed to 20  $\mu$ M A $\beta$ P[1–40]. An exception to this was the N-channel antagonist diltiazem, which consistently reduced A $\beta$ P[1–40] cytotoxicity. There is experimental evidence to support the view that A $\beta$ P-induced Ca<sup>2+</sup> influx in neurons is via a protein-kinase-phosphorylated VSCC. For example, A $\beta$ P treatment increased L-type Ca<sup>2+</sup> channel phosphorylation, which was unaffected by the protein kinase A inhibitor H89 but was reduced by the mitogen-activated protein (MAP) kinase inhibitor PD98059 (Ekinci *et al.*, 1999). They proposed that redirection, rather than increased activation, of MAP kinase activity mediates A $\beta$ P cytotoxicity. They used pharmacological agents to deduce a role for L-type VSCC in A $\beta$ P-induced changes in [Ca<sup>2+</sup>]<sub>i</sub>. Phenothiazines, which are a class of neuroleptic agents (e.g. chlorpromazine, promethazine, and trifluoperazine), were effective in blocking A $\beta$ P[25–35] at micromolar concentrations. Nimodipine also acted to reduce the increase in Ca<sup>2+</sup> uptake caused by A $\beta$ P[25–35]. The results suggest that phenothiazines lessen A $\beta$ P[25–35] cytotoxicity, possibly by reducing Ca<sup>2+</sup> intake through L-type Ca<sup>2+</sup> channels (Ueda *et al.*, 1997). Silei *et al.* (1999) reported that activation of microglial cells by A $\beta$ P, like PrP[106–126], raises [Ca<sup>2+</sup>]<sub>i</sub> through L-type VSCC.

Ekinci *et al.* (2000) established the sequence of the major steps in A $\beta$ P toxicity in cultured cortical neurons and SH-SY-5Y neuroblastoma cells. They reported that Ca<sup>2+</sup> influx, which represents the initial event—since it is known that Ca<sup>2+</sup> chelation prevents all subsequent events—is followed by oxidative stress and phosphorylation. They ascertained that Ca<sup>2+</sup> influx is followed by oxidative stress—since vitamin E prevented ROS generation and apoptosis—but did not prevent intracellular Ca<sup>2+</sup> accumulation or tau phosphorylation. In contrast W7, which inhibits tau phosphorylation, did not prevent A $\beta$ P-induced Ca<sup>2+</sup> influx, ROS generation, or apoptosis.

The role of A $\beta$ P in modulation of the NMDA receptors' Ca<sup>2+</sup>-permeable channels in neurons has also been suggested. Brorson *et al.* (1995) found that A $\beta$ P[25–35] or A $\beta$ P[1–40], applied to rat hippocampal neurons in culture, caused reversible and repeatable increases in [Ca<sup>2+</sup>]<sub>i</sub> and induced bursts of excitatory potentials and action potential firing in individual neurons. The increase in [Ca<sup>2+</sup>]<sub>i</sub> was very likely mediated by A $\beta$ P modulation of more than one Ca<sup>2+</sup> transport system. This is because

the A $\beta$ P[25–35]-induced [Ca<sup>2+</sup>]<sub>i</sub> elevations and electrical activity were enhanced by the removal of extracellular Mg<sup>2+</sup>, and they could be blocked by tetrodotoxin, by NMDA receptor antagonists, and by the L-type Ca<sup>2+</sup> channel antagonist nimodipine. These excitatory responses and elevations in [Ca<sup>2+</sup>]<sub>i</sub> were not observed in cerebellar neuron cultures in which inhibitory synapses predominate. A $\beta$ P[25–35] did not enhance kainate- or NMDA-induced currents. These findings could suggest that A $\beta$ P effects are localized or tissue-specific.

Recently, MacManus *et al.* (2000) found that A $\beta$ P[1–40] causes a significant increase in <sup>45</sup>Ca<sup>2+</sup> influx into rat cortical synaptosomes via activation of L- and N-type voltage-dependent Ca<sup>2+</sup> channels and also increased the amplitude of N- and P-type Ca<sup>2+</sup> channel currents recorded from cultured cortical neurons. On the other hand, they found that interleukin-1 $\beta$  reduced the <sup>45</sup>Ca<sup>2+</sup> influx into cortical synaptosomes and inhibited Ca<sup>2+</sup> channel activity in cultured cortical neurons. They proposed that since the stimulatory effects of A $\beta$ P on Ca<sup>2+</sup> influx could be blocked by interleukin-1 $\beta$ , interleukin-1 $\beta$  modulates neuronal responses to A $\beta$ P by regulating Ca<sup>2+</sup> homeostasis.

**K<sup>+</sup> Channels.** The cytotoxic effects of A $\beta$ P could also be mediated via alterations in the K<sup>+</sup> channel activity in neurons. K<sup>+</sup> channels are important in the function of neurons. They play a significant role in learning and memory as well as in growth and differentiation (see Alkon *et al.*, 1991; Etcheberrigaray, 1994a,b). A $\beta$ P-induced changes in K<sup>+</sup> channel properties will modify the membrane potential on which not only Ca<sup>2+</sup> influx and Ca<sup>2+</sup> signaling are dependent but also several other voltage-dependent membrane properties. Recent evidence implicate the up-regulation of outward K<sup>+</sup> current in mediating several forms of neuronal apoptosis (Colom *et al.*, 1998; Yu *et al.*, 1998). The effects of A $\beta$ P on K<sup>+</sup> channels have been examined in various cell types (Table III). Etcheberrigaray *et al.* (1994) found that the treatment of fibroblasts with A $\beta$ P (10 nM) induced the same 113-pS K<sup>+</sup> channel dysfunction previously shown to occur in fibroblasts from patients with AD. They also found that tetraethylammonium (TEA)-induced increase of [Ca<sup>2+</sup>]<sub>i</sub>, a response that depends on functional 113-pS K<sup>+</sup> channels, was also eliminated or markedly reduced by 10 nM A $\beta$ P. On the other hand, the increased [Ca<sup>2+</sup>]<sub>i</sub> induced by high concentrations of [K<sup>+</sup>]<sub>o</sub> and 166-pS K<sup>+</sup> channels were unaffected by 10 nM A $\beta$ P. The difference in K<sup>+</sup> channel responses to A $\beta$ P have been attributed to a specific modification in a regulatory protein, Cp20, a GTP-binding protein that is a member of the ADP-ribosylation factor family, of the 113-pS K<sup>+</sup> channel (Kim *et al.*, 1995). In contrast to the above findings regarding the alterations of Ca<sup>2+</sup> homeostasis in fibroblasts and T-lymphocytes of patients with AD, Cohen *et al.* (1996) reported that data, obtained by means of the whole-cell patch-clamp configuration on freshly isolated T lymphocytes, indicate that K<sup>+</sup> channels of these cells do not present any functional deficit in AD, and A $\beta$ P does not mediate an alteration of their currents.

Jalonen *et al.* (1997) studied in vitro effects of A $\beta$ P[1–40] and A $\beta$ P[25–35] on rat cortical astrocyte ion channel activity. In outside-out patches, increased intrinsic K<sup>+</sup> (120–160 pS) activity was detected with 10–100 nM A $\beta$ P[1–40]. With large amounts (20  $\mu$ M) of A $\beta$ P[1–40], the activity of a formed giant channel (>700 pS) is additionally observed. Unlike the studies of Yu *et al.* (1998) and Colom *et al.* (1998) (see later), where A $\beta$ P effects were deduced from whole-cell recording of currents after chronic

exposure of neuronal cultures to A $\beta$ P, Good *et al.* (1996) examined the acute response of rat hippocampal neurons, measured using whole-cell voltage-clamp techniques, directly after the application of synthetic A $\beta$ P. They found that pulse application of A $\beta$ P caused a reversible voltage-dependent decrease in membrane conductance. A $\beta$ P selectively blocked ( $K_i < 10 \mu\text{m}$ ) the voltage-gated fast-inactivating K $^+$  current. A $\beta$ P also blocked the delayed rectifying current, but only at the highest concentration tested. The response was independent of aggregation state or peptide length. It was suggested that the mechanism of A $\beta$ P-induced block of the fast-inactivating current involves a reversible binding of A $\beta$ P to the closed channels and thus prevents their opening. The physiological implication of such A $\beta$ P-induced blockage of the fast-inactivating K $^+$  channels is that A $\beta$ P will cause prolonged cell depolarization, thereby increasing Ca $^{2+}$  influx and thus modifying Ca $^{2+}$  homeostasis and signaling. A simulated mathematical model shows that this block could result in increased intracellular Ca $^{2+}$  levels and membrane excitability (Good *et al.*, 1996; Good and Murphy, 1996).

Yu *et al.* (1998) found that A $\beta$ P[25–35] and A $\beta$ P[1–42] enhanced the outward delayed rectifier K $^+$  current,  $I_K$ , in murine-cultured cortical neurons, shifting its activation voltage relationship toward hyperpolarized levels and increasing maximal conductance. However, it did not affect the transient K $^+$  current ( $I_A$ ), charybdotoxin-sensitive BK current or the inward rectifying current. Reducing the  $I_K$  current by adding TEA or increasing  $[K^+]_o$  also attenuated the A $\beta$ P-induced neuronal death. This is in contrast to the observations of Chi *et al.* (1999) who found that prevention of A $\beta$ P-induced endothelial damage was reversed by TEA. In addition, Good *et al.* (1996) observed a voltage-dependent blockade of  $I_A$  in rat hippocampal neurons when A $\beta$ P was added in pulse applications. At high concentrations A $\beta$ P also blocked  $I_K$ , as opposed to increasing conductance levels. Good *et al.* (1996) found the blockade by A $\beta$ P to be independent of aggregation state, whereas channel formation is thought to be dependent on aggregation (Hirakura *et al.*, 1999). Good and Murphy (1996) used mathematical modeling to demonstrate that blockade of  $I_A$  could explain the increase in  $[Ca^{2+}]_i$  observed with addition of A $\beta$ P. A $\beta$ P-induced depolarization may enhance the flow of Ca $^{2+}$  through depolarization-activated Ca $^{2+}$  channels. In addition, it is very likely that some A $\beta$ P-induced enhancement in K $^+$  currents are indirect effects that could be mediated via an increase in Ca $^{2+}$ -activated channels. To dissect these effects merits evaluation.

Several studies point to a protective role for K $^+$  channel openers (KCOs, e.g. cromakalim, nicorandil, and pinacidil), in protecting neuronal cells against degeneration (Chi *et al.*, 1999; Goodman and Mattson, 1996; Heurteaux *et al.*, 1993). It was suggested that the mechanism of the neuroprotective effects involves the opening of ATP-sensitive K $^+$  channels, since glipizide, a specific blocker of that type of channel, abolished the beneficial effects of KCOs (Heurteaux *et al.*, 1993). It is assumed KCOs-induced channel opening would cause the hyperpolarization of the plasma membrane and therefore reduce Ca $^{2+}$  influx. Also, Goodman and Mattson (1996) reported that the KCOs, such as diazoxide, levochromakalim and pinacidil can protect cultured rat hippocampal neurons against oxidative injury induced by exposure to FeSO $_4$  and A $\beta$ P against excitotoxic injury. However, they suggested that, in addition to KCOs-induced K $^+$  channel activation, KCOs suppressed the generation of

Table III. Effects of APP and A $\beta$ P on K<sup>+</sup> Channels

Preparation membrane, and tissue types	A $\beta$ P type and concentration, or IC <sub>50</sub>	Ion channel type	Methods	A $\beta$ P-induced effects	Mechanism of inhibition	Reference
Hybrid SNS6 cells	1–10 $\mu$ M A $\beta$ P[1–40]	K <sup>+</sup> channels	Patch-clamp technique (whole-cell)	Increased K <sup>+</sup> current density. Enhanced $I_K$	Cell death prevented by TEA and high [K <sup>+</sup> ] <sub>o</sub> .	Colom <i>et al.</i> (1998)
Cultured mouse cortical neurons	20 $\mu$ M A $\beta$ P-[1–42] and A $\beta$ P[25–35] (paired)	Voltage-gated K <sup>+</sup> channels	Patch-clamp technique (whole-cell)	Induced a persistent [K <sup>+</sup> ] <sub>out</sub> current at $V_{mems}$ positive to –100 mV, reflecting a shift in the $I_k$ $I$ - $V$ relationship to hyperpolarized potentials. [K <sup>+</sup> ] <sub>out</sub> increase not due to decrease in inward Na <sup>+</sup> or Ca <sup>2+</sup>	Extracellular K <sup>+</sup> or TEA prevented cell death despite blockage of voltage-gated Ca <sup>2+</sup> channels with Ca <sup>2+</sup> channel antagonists (Gd <sup>3+</sup> , nifedipine). No neuroprotection with the Cl <sup>-</sup> channel antagonist ACA (500 $\mu$ M)	Yu <i>et al.</i> (1998)
Rat cortical astrocytes	10 nM A $\beta$ P[1–40]. Incubated overnight. 100 nM A $\beta$ P[1–40] added in bath or recording pipette during experiment	Large conductance K <sup>+</sup> channels (120–160 pS). Large conductance Cl <sup>-</sup> channels (200–400 pS)	Patch-clamp technique (whole-cell)	Continuous activity of K <sup>+</sup> channels with multiple fast-flickering openings recorded for incubated 10 nM A $\beta$ P[1–40]. 100 nM A $\beta$ P[1–40] increased $F_o$ of large conductance K <sup>+</sup> channel (120–160 pS). Increase in the activity of 200–400-pS swelling-activated Cl <sup>-</sup> channel. At larger concentrations of A $\beta$ P[1–40] (20 $\mu$ M), giant channels (>700 pS) formed	200–400-pS Cl <sup>-</sup> channel inhibited by L-644 (anion transport inhibitor)	Jalonen <i>et al.</i> (1997)
Isolated T-lymphocytes	2.5 $\mu$ M of A $\beta$ P [25–35]. Incubation of T-lymphocytes and A $\beta$ P[1–40] (0.1, 1, and 2 $\mu$ M)	K <sup>+</sup> channels	Patch-clamp technique (whole-cell)	No effect on $K(v)$ conductance after addition of A $\beta$ P[25–35]. No effect on cell growth and expression of K <sup>+</sup> channels for A $\beta$ P[1–40]. [Ca <sup>2+</sup> ] <sub>i</sub> disturbances in T-lymphocytes of AD patients not mediated by altered K <sup>+</sup> channel function		Cohen <i>et al.</i> (1996)

Rat hippocampal neurons	Pulse application of A $\beta$ P[1-30], A $\beta$ P[1-40], A $\beta$ P[1-28]	Voltage-gated K <sup>+</sup> channels	Patch-clamp technique (whole-cell)	Blocks fast-inactivating K <sup>+</sup> channel and delayed-rectifying K <sup>+</sup> channel (less potency). Gating properties and kinetics unchanged	Fast-inactivating K <sup>+</sup> channels blocked, causing Ca <sup>2+</sup> influx, intracellular accumulation, and finally neural dysfunction and death	Good and Murphy (1996)
Rat cortical neurons	10 nM-1 mM A $\beta$ P[25-35]	Irreversible nonselective channels	Patch-clamp technique (whole-cell)	Increased membrane conductance 3.6 times. Conductance increase not concentration dependent, but time lag to <i>I</i> increase is. <i>I</i> - <i>V</i> relationships almost linear. <i>E</i> <sub>rev</sub> ≈ 0 mV	NMG, isothionate <sup>-</sup> , and Cs <sup>+</sup> had no effect	Furukawa <i>et al.</i> (1994)
Cultured embryonic hippocampal neurons	1 nM sAPP695 applied	High conductance (~240 pS) K <sup>+</sup> channel	Whole-cell and single-cell patch-clamp	Reversible hyperpolarization. Action potential frequency reduced (current clamp conditions). Outward current from -80 to -20 mV, with a reversal potential of -85 mV (voltage clamp conditions). Increased <i>P</i> <sub>o</sub> of a high conductance K <sup>+</sup> channel (single channel recording)	sAPP-induced outward current completely blocked by TEA (10 mM) and CTX (30 nM). No blockage by apamin	Furukawa <i>et al.</i> (1996)

peroxides induced by  $\text{FeSO}_4$  and  $\text{A}\beta\text{P}$ . This suggestion was confirmed by the finding that KCOs were effective in protecting neurons against oxidative insults in the presence of the  $\text{K}^+$  channel blockers, glibenclimide and 4-aminopyridine. Chi *et al.* (1999) investigated the effects of KCOs on endothelial damage induced by  $\text{A}\beta\text{P}$ . Pretreatment with KCOs reduced the effects of  $\text{A}\beta\text{P}$ , such as enhanced vasoconstriction and diminished vasodilation. However, it appears that other  $\text{K}^+$  channels are involved. Both  $\text{Ca}^{2+}$ -activated channels and  $\text{K}_{\text{ATP}}$  channel openers achieved this effect. In addition, unlike the findings with the ATP-sensitive  $\text{K}^+$  channel ( $\text{K}_{\text{ATP}}$  channel) blockers, glibenclimide and 4-aminopyridine,  $\text{TEA}^+$ , (a  $\text{K}^+$  channel blocker) reversed the endothelial damage induced by  $\text{A}\beta\text{P}$ . In general, the aforementioned studies point to the potential of KCOs in the treatment of neurodegenerative and cerebrovascular disease.

*$\text{Cl}^-$  and  $\text{Na}^+$  Channels.* Sodium and chloride channels play important roles in the function of the brain. There are fewer studies on the effect of  $\text{A}\beta\text{P}$  or APP on  $\text{Na}^+$  and  $\text{Cl}^-$  transport systems. Jalonen *et al.* (1997) studied in vitro effects of  $\text{A}\beta\text{P}[1-40]$  and  $\text{A}\beta\text{P}[25-35]$  on rat cortical astrocyte ion channel activity. In outside-out patches,  $\text{Cl}^-$  channel (200–400 pS) activity was detected with 10–100 nM  $\text{A}\beta\text{P}[1-40]$ . These findings were taken to suggest effects on the cytoskeleton, e.g. rearrangement or breakage of actin filaments. It was additionally observed that with large amounts (20  $\mu\text{M}$ ) of  $\text{A}\beta\text{P}[1-40]$ , ion current activity of multimers formed giant channels (>700 pS). Sawada and Ichinose (1996) suggested that  $\text{A}\beta\text{P}$  may increase neuronal excitability by inhibiting GABA-induced  $\text{Cl}^-$  current in the neurons of mammalian central nervous system. This suggestion was based on their findings which show that in neurons (R9 and R12) of *Aplysia kurodai*, focal application of 100 nM  $\text{A}\beta\text{P}[1-40]$  reversibly reduced the GABA-induced hyperpolarization at resting membrane potential and the GABA-induced  $\text{Cl}^-$  current. The role of two  $\text{A}\beta\text{P}$  fragments was also examined. It was found that bath-applied 100 nM  $\text{A}\beta\text{P}[1-40]$  and  $\text{A}\beta\text{P}[25-35]$ , but not  $\text{A}\beta\text{P}[1-16]$ , inhibited the GABA-induced  $\text{Cl}^-$  current.

Little work has been done to examine the effects of  $\text{A}\beta\text{P}$  on  $\text{Na}^+$  channels and the reported findings have been merely passing observations in studies where the primary focus was another transport system. Nonetheless, it has been noted that TTX-sensitive sodium currents were not affected by  $\text{A}\beta\text{P}$  (see Colom *et al.*, 1998). This may be of importance since it appears that there is evidence to suggest that TTX-sensitive  $\text{Na}^+$  channels may play a role in APP release as discussed above (Nitsch *et al.*, 1993). A lack of  $\text{A}\beta\text{P}$  effects on  $\text{Na}^+$  channels is in agreement with continuous release of the sAPP.

### Amyloid-Formed Channels

Hirakura and Kagan (1999) detailed all known amyloid-forming peptides and their associated pathologies. Some of these amyloid-forming peptides have already been shown to form ion channels. These include  $\text{A}\beta\text{P}$ -formed channels (see table IV, Kourie and Shorthouse, 2000); prion peptide PrP [106–126]-formed channels (Lin *et al.*, 1997); amylin-formed ion-permeable channels (Kourie and Culverson, 2000; Mirzabekov *et al.*, 1994) and C-type natriuretic peptide-formed channels (Kourie, 1999b–d; Kourie and Rive, 1998). According to the “ion channel hypothesis,” reviewed

by Pollard *et al.* (1993, 1995), A $\beta$ P exerts its cytotoxic effects by forming ion channels that cause changes in Ca<sup>2+</sup> homeostasis and Ca<sup>2+</sup> signaling and consequently cell death. There is early evidence, though difficult to ascertain, for ion channel formation in neurons and red blood cells by A $\beta$ P. The difficulty in the interpretation of these studies is that changes in A $\beta$ P-induced membrane conductance and ionic currents cannot be distinguished with great certainty from those that arise from endogenous ion channels. For example, Furukawa *et al.* (1994) examined the early effect (in seconds) of A $\beta$ P in neurons dissociated from rat cortex by using the nystatin perforated patch-clamp technique. They found that A $\beta$ P at concentrations >10 nM induced an irreversible, slow nonselective inward current associated with an increase in membrane conductance. It was suggested that A $\beta$ P binds to the membrane and forms ion pores; however, no single channels were analyzed and thus modification of intrinsic ion channels cannot be ruled out. Patch-clamp experiments show that exposure of hNT cells, which display neuronlike Ca<sup>2+</sup> channel activation, to A $\beta$ P[25–35] fragment induces (in seconds) large and irreversible inward Ca<sup>2+</sup> currents at –80 mV in whole-cell mode, with a linear current–voltage relationship (Sanderson *et al.*, 1997). The currents are large and stable, and are blocked by Al<sup>3+</sup> but not by Cd<sup>2+</sup>. Filtration, which removes a peptide aggregate from the A $\beta$ P[25–35] solution, abolishes the inward current and the residual soluble peptide does not induce Ca<sup>2+</sup> currents. They proposed that the initial step of the neurotoxic effect of A $\beta$ P[25–35] may be due to the insertion of the aggregated peptide into the cellular membrane as a Ca<sup>2+</sup>-carrying ionophore. Both A $\beta$ P[1–42] and A $\beta$ P[25–35] caused red blood cell lysis, and both were inhibited by aggregation inhibitor Congo Red. The A $\beta$ P[25–35] effects were rapid and mediated via free radical increase, whereas the A $\beta$ P[1–42] effects were slower and could be attenuated by antioxidants (Mattson *et al.*, 1997). However, it cannot be ruled out that the observed currents or cell lysis are due to activation of an intrinsic transport system or that the residual soluble peptide may have effects on other transport systems, which may themselves be affected before A $\beta$ P aggregation. The above studies are supported by studies involving liposomes. For example, it has been found that A $\beta$ P[25–35] increased dye leakage from liposomes composed of phosphatidylcholine (PC) and (PE) phosphatidylethanolamine, but had little effect on phosphatidylserine (PS) liposomes and less on sphingomyelin liposomes (Hirakura *et al.*, 1998). This effect is thought to be due to a nonspecific membrane perturbation. Further studies are needed to clarify the effects of APP and A $\beta$ P on lipid properties, e.g. structure and fluidity.

#### *A $\beta$ P-Formed Channels*

In detailed ion flux experiments on vesicles reconstituted with immunofluorescence-labeled A $\beta$ P, it was found that A $\beta$ P forms a <sup>45</sup>Ca<sup>2+</sup> transport system (Lin *et al.*, 1999; Rhee *et al.*, 1998). This transport system was inhibited by a monoclonal antibody raised against the N-terminal region of A $\beta$ P, Tris, and Zn<sup>2+</sup>. They also noted that this transport system was not affected by reducing agents Trolox and dithiothreitol. This finding was taken to suggest that oxidation of A $\beta$ P and its environment were not directly involved in the A $\beta$ P-mediated Ca<sup>2+</sup> uptake. The lipid bilayer and

patch-clamp techniques have been used to confirm that A $\beta$ P fragments form single ion channels (Arispe *et al.*, 1993a,b, 1994, 1996; Durell *et al.*, 1994; Hirakura *et al.*, 1999, 2000; Hirakura and Kagan, 1999; Kawahara *et al.*, 1997; Mirzabekov *et al.*, 1994; Pollard *et al.*, 1995). Table IV indicates that one of the most apparent features of these A $\beta$ P-formed channels is that they differ in those properties (conductance, kinetics, selectivity, and pharmacological activity) which are often used for channel classification.

**Conductance.** The A $\beta$ P-formed channels are heterogeneous and have a wide range of subconductance states. Channel conductances as high as 5 nS have been noted (Durell *et al.*, 1994; Pollard *et al.*, 1995). Small channels with conductance of 10 pS have also been recorded (Hirakura *et al.*, 1999). Arispe *et al.* (1994) noted spontaneous transitions between multiple conductance levels in both the 40–400 pS and the 400–4,000 pS ranges. Kawahara *et al.* (1997) observed complete closure from a range of different conductance levels, indicating the presence of a single multi-conductance channel. Arispe *et al.* (1996) reported an A $\beta$ P[1–40]-formed channel that usually open to the maximal conductance (110 pS) with infrequent short-lived (spiky) closure to subconductances. Zn<sup>2+</sup> induces a significant change in the kinetics of this channel, with high frequency transitions between lower levels of conductance observed, the most common being 82, 44, and 19 pS.

**Cation/Anion Permeability and Selectivity Sequence.** Arispe *et al.* (1993a,b) observed that a channel of conductance 325 pS in symmetrical 40 mM KCl was found to have a permeability sequence of  $P_{Cs}:P_{Li}:P_{Ca}:P_{K}:P_{Na} = 1.0:0.63:0.60:0.60:0.46$ . This sequence accords with that of known Ca<sup>2+</sup> channels. The A $\beta$ P[25–35]-formed channel showed a permeability ratio of  $P_{Ca^{2+}}:P_{K^+}:P_{Na^+}:P_{Cl^-} = 5.4:1.6:1.4:1$ . However, Ca<sup>2+</sup> channels are typically impermeable to monovalent cations in the presence of micromolar concentrations of Ca<sup>2+</sup>. The A $\beta$ P[1–40]-formed channel, however, is permeable to monovalent cations in the presence of 1 mM Ca<sup>2+</sup>, though blockade is achieved at 10 mM Ca<sup>2+</sup> (Arispe *et al.*, 1993a). This quantitative difference may or may not be indicative of a difference in channel mechanism. The ratio of permeabilities of monovalent cations through a large A $\beta$ P channel was  $P_{K}:P_{Na}:P_{Cs}:P_{Li} = 1.0:0.21:0.11:0.03$  (Kourie, unpublished).

**Kinetics.** The kinetic behavior of the A $\beta$ P-formed channels is observed to differ widely. Kinetic types range from high frequency flickering between subconductance levels to conversions between long-lasting levels (Arispe *et al.*, 1994). These differences suggest that the A $\beta$ P incorporated into the membrane may have different configurations. Channel gating, evaluated in terms of the fraction of time spent at any one level of conductance, seems to be insensitive to the transmembrane potential in the presence of monovalent cations (Arispe *et al.*, 1996). However, voltage-dependent channel gating can be observed where Ca<sup>2+</sup> and Zn<sup>2+</sup> can enter the pore.

The kinetics of the large K<sup>+</sup> channel conductance apparently random, with levels of 1.8, 2.3, 2.5, and 4.2 nS having corresponding  $P_0$  values of 0.32, 0.30, 0.05, and 0.33, respectively (Arispe *et al.*, 1993b). Zn<sup>2+</sup> increases the frequency of transitions between certain subconductance levels. Also, a rapid flickering can occur between two nS subconductances spontaneously (Arispe *et al.*, 1993b). Thus, the differences between channel gating mechanisms are not necessarily irreversible once the protein has incorporated.

**Table IV.** Summary of the Properties of Heterogenous Ion Channels Formed by APP Proteolytic Fragments (CT105 and A $\beta$ P)

Peptide, ionic selectivity, and method	Single channel conductance (pS), current-voltage ( $I-V$ ), and slope conductance at [ion] <i>cis/trans</i> or bath/pipette (mM)	Open probability ( $P_o$ )	Mechanism of inhibition by organic and inorganic blockers	Reference
Serum amyloid A cation selective K <sup>+</sup> : Cl <sup>-</sup> = 2.9 : 1.0	10–400 pS voltage-independent		Channel blocked by [Zn <sup>2+</sup> ] Channel formation inhibited by [Congo Red] <sub><i>cis</i></sub>	Hirakura and Kagan (1999)
A $\beta$ P [25–35] cation selective	10–400 pS (100/100 KCl)		14 $\mu$ M [Congo Red] <sub><i>cis</i></sub> blocked channel formation	Hirakura <i>et al.</i> (1999)
A $\beta$ P [1–40]	10–2000 pS voltage-independent (100/100 KCl)		No channel block with Tromethamine (Tris)	
A $\beta$ P [1–42] slightly cation selective (permeable to K <sup>+</sup> , Na <sup>+</sup> , Cl <sup>-</sup> , Mg <sup>2+</sup> , and Ca <sup>2+</sup> )	10–2000 pS (100/100 KCl) voltage-independent, linear and slope conductance of 1 nS		250 $\mu$ M [Zn <sup>2+</sup> ] <sub><i>cis</i></sub> block is voltage-independent	
Azolectin bilayer	Model channel 34-pS conductance similar to that of A $\beta$ P [1–40]		No channel block with Al <sup>3+</sup>	
A $\beta$ P [1–40] cation-selective channels	Spontaneous conductance changes; complete closures of 0 mV (140/140 CsCl)	$P_o \sim 0.8$ at –40 mV–40 mV Zn <sup>2+</sup> ( $\leq 250 \mu$ M) changed the $P_o$ from $\sim 0.05$ at –40 mV to 0.17 at 40 mV	Zn <sup>2+</sup> -induced voltage-dependent block. 250 $\mu$ M [Zn <sup>2+</sup> ] <sub><i>cis</i></sub> reduced current amplitude and increased the frequency transition between 0.69, 1.0, and 1.85 pA	Kawahara <i>et al.</i> (1997)
A $\beta$ P channel formation deposited on the cell surface of cultured neurons	Multilevel channel activity observed: 1.0, 1.7, 2.6, 4.1, 5.9, 9.3, and 12.8 pA. $E_{rev} \sim 16$ mV (140/70 CsCl)			
A $\beta$ P [1–40] single multiconductance channel	110 pS (40/40 CsCl) 165 pS at –1 mV, 1.9 nS at –10 mV (140/140 KCl)	Infrequent, fast transitions to basal level of current	At low conductance <400 pS, Increased $F_o$ to >88 pS Decreased $P_o$ for the 225-pS conductance (0.5 control) and Zn <sup>2+</sup> = 0.14 (EC <sub>50</sub> 300) Large conductance (>400 pS) A $\beta$ P channel blocked by mM Zn <sup>2+</sup> but to a lesser extent than the <400 pS channels at any given Zn <sup>2+</sup> concentration	Arispe <i>et al.</i> (1996)
Fused unilamellar liposomes containing A $\beta$ P				(Continued)

Table IV. (Continued)

Peptide, ionic selectivity, and method	Single channel conductance (pS), current-voltage ( $I-V$ ), and slope conductance at [ion] <i>cis/trans</i> or bath/pipette (mM)	Open probability ( $P_o$ )	Mechanism of inhibition by organic and inorganic blockers	Reference
A $\beta$ P [1-40]	Current-voltage relationship linear. Slope conductance: 4.2 nS (200 mM K <sup>+</sup> and 200 $\mu$ M Ca <sup>2+</sup> ) Rapid transitions between small (pS range) and large conductance levels (nS range)	High frequency flickering between two conductance levels Frequent interconversions between stable and long lasting conductance levels	10 $\mu$ M [Al <sup>3+</sup> ] blocked conductance Large channels (>400 pS) blocked completely by 1-2 mM Tris; giant channels (>400 pS) minimally affected 100 $\mu$ M [nitrendipine] <sub>cis</sub> or 100 $\mu$ M [nitrendipine] <sub>trans</sub> failed to affect conductivity 100 $\mu$ M Cognex® ineffective as blocking agent of Cs <sup>+</sup> -permeation of A $\beta$ P [1-40] channels "Giant" channel less susceptible to blockage by Tris Channel blocked by [Zn <sup>2+</sup> ] Channel formation inhibited by [Congo Red] <sub>cis</sub>	Arispe <i>et al.</i> (1994)
A $\beta$ P [1-40] cation selective	40-400 pS and 0.4-5.0 nS	Channels open at negative voltages and closed at positive voltages		Durell <i>et al.</i> (1994)
A $\beta$ P [25-35] voltage-dependent channels Ca <sup>2+</sup> : K <sup>+</sup> : Na <sup>+</sup> : Cl <sup>-</sup> = 5.4 : 1.6 : 1.4 : 1	10-400 pS voltage-dependent			Mirzabekov <i>et al.</i> (1994)
Solvent-free planar phospholipid bilayers, Soybean phospholipids or DGPC. Montal-Mueller technique				
Cation selective channel $P_K/P_{Cl} \sim 11$ $P_{Cs}/P_{Li} = 0.6$ $P_{Cs}/P_{Li} \sim 1.6$ $P_K/P_{Na} = P_{Cl}/P_{Na} = 1.3$ . $P_{Cs} > P_{Li} > P_{Ca} \geq P_K > P_{Na}$	Multiple conductance levels. Linear $I-V$ . $E_{rev}$ 0 in symmetric KCl. $E_{rev}$ 8.5 mV in asymmetric 40/60 KCl	Open-time probabilities for principal conductance states similar; $P_o$ for conductance levels 1.8, 2.3, 2.5, and 4.2 nS were 0.32, 0.30, 0.05 and 0.33, respectively	Blocked by 25 mM Tris when conductance <400 pS; less effective in nS range Increasing [Ca <sup>2+</sup> ]: 1-10 mM in <i>cis</i> blocks Cs <sup>+</sup> flow (voltage-dependent blockade of channel activity)	Arispe <i>et al.</i> (1993a,b) Arispe <i>et al.</i> (1994)

<p>A<math>\beta</math>P incorporated into PS liposomes and liposomes containing A<math>\beta</math>P added to <i>cis</i> solution</p>	<p>Cs<sup>+</sup> charge carrier at positive potentials: conductance ~83 pS cf. ~206 pS in symmetrical 75 mM CsCl</p>	<p>Blocked by 10 or 20 <math>\mu</math>M Al<sup>3+</sup> Blocked by 1 mM Al<sup>3+</sup> was rapid and persisted</p>
<p>105-amino-acid C-terminal fragment <math>\beta</math>APP (CT105) incorporated into artificial lipid membranes. Ca<sup>2+</sup>-permeable cation selective channels. <math>P_{K^+}/P_{Cl^-} = 10.2</math></p>	<p>325 pS in symmetrical 40 KCl and 346 pS in asymmetrical (40/60 KCl) solutions Single channel conductance 120 pS. Linear <i>I-V</i> relationship of single channels Membrane conductance increased with increased concentration of CT105</p>	<p>Reversibly blocked by <math>\mu</math>M [Cu<sup>2+</sup> or Cd<sup>2+</sup>]<sub>cis</sub></p>
<p>Selectivity: Ca<sup>2+</sup>:Na<sup>+</sup>:K<sup>+</sup>:Rb<sup>+</sup>:Li<sup>+</sup>:Cs<sup>+</sup>:Mg<sup>2+</sup> = 1.61:1.40:1:0.79:0.58:0.50:0.41 (different sequence from A<math>\beta</math>P)</p>	<p>0.85 <math>\leq P_o \leq 0.99</math>, from -80 to +80 mV</p>	<p>Kim <i>et al.</i> (1999)</p>
<p><i>Xenopus</i> oocytes voltage clamp</p>	<p>Membrane conductance increased with increased concentrations of CT105. Constant, inward membrane current, with late-onset oscillatory component (due to Ca<sup>2+</sup>-dependent Cl<sup>-</sup> currents)</p>	<p>Fraser <i>et al.</i> (1996)</p>
<p>CT105. Nonselective ion currents with concentration threshold of 100–200 nM</p>		

Kawahara *et al.* (1997) found that the gating of a large multi-conductance channel was voltage-insensitive, although this was altered in the presence of  $Zn^{2+}$  and other divalent cations. Arispe *et al.* (1996) reported a channel usually open to the maximal conductance (110 pS) with infrequent short-lived (spiky) closure to subconductances. When  $Zn^{2+}$  was added, a significant change in kinetics was noted, with high frequency transitions between lower levels of conductance observed. When  $Zn^{2+}$  was removed from the trans side, the effects were reversed. When added to giant channels (maximal conductance  $>400$  pS),  $500 \mu M Zn^{2+}$  reduced conductance from 4.7 to 197 pS, several minutes after application. The frequency of transitions between sublevels did appear to increase.

*Pharmacology.* Because the cytotoxic action of A $\beta$ P on target cells in the brain might be attributed to the formation of A $\beta$ P[1–42]-formed channels, blocking these channels might be therapeutically useful. Some of these probing drugs discussed are also useful for the structure–function relationship of the channel. Ion channel blockers, organic and inorganic, have also been used to examine the inhibition of the A $\beta$ P-formed channel.

**INORGANIC BLOCKERS:** Zinc is known to interact strongly with A $\beta$ P, and at high concentrations it precipitates out A $\beta$ P and thus  $Zn^{2+}$  may have a role in plaque formation.  $Zn^{2+}$  also inhibits current through A $\beta$ P-formed channels (Arispe *et al.*, 1996; Kawahara *et al.*, 1997). The A $\beta$ P-formed channels differ in their sensitivity to  $Zn^{2+}$ . At conductance levels below 400 pS the blockade was [ $Zn^{2+}$ ]-dependent (Arispe *et al.*, 1996). For A $\beta$ P channels at conductance greater than 400 pS, millimolar quantities of  $Zn^{2+}$  are required to produce channel block.  $Zn^{2+}$  at a concentration of 50–500  $\mu M$  can block the channel from either side, and this block is reversed by *o*-phenanthroline (a  $Zn^{2+}$  chelator) added to the side containing  $Zn^{2+}$ . The fast blocking events could be explained by low affinity association between  $Zn^{2+}$  and A $\beta$ P-formed channels. The low affinity binding may induce rapid rearrangements of the molecules, forming part of the channel aggregate (Durell *et al.*, 1994). These conformational changes may cause the observed flickering in the channel activity. It is thought that the interaction of  $Zn^{2+}$  with A $\beta$ P-formed channels must occur in a high dielectric constant region, probably at the entrance of the channel, where the electrical field is relatively constant. The A $\beta$ P-formed channel is asymmetric. Arispe *et al.* (1996) found that  $Zn^{2+}$  modulated the gating and conductance of an A $\beta$ P[1–40]-formed channel only from one side of the channel. On the other hand, Kawahara *et al.* (1997) found that  $Zn^{2+}$  affected current and kinetics from both sides of the channel. However, the degree of blockade induced by  $Zn^{2+}$  is dependent on the direction of cation flow and whether it was added to the same side of the membrane as A $\beta$ P. It is thought that  $Zn^{2+}$  binds differentially to two or three binding sites near the entrance of an asymmetric channel (Arispe *et al.*, 1996; Bush *et al.*, 1994). The A $\beta$ P[25–35]-formed channel is also blocked reversibly with micromolar concentrations of  $Cu^{2+}$  or  $Cd^{2+}$  (Mirzabekov *et al.*, 1994). Aluminium in the form of  $AlCl_3$  or  $Al_2(SO_4)_3$  at micromolar concentrations inhibits A $\beta$ P-formed channels irreversibly. The blockade of current in the A $\beta$ P[1–40]-formed channels by  $Al^{3+}$  is like that induced by tromethamine (Tris base), dose- and voltage-dependent and suggests a direct interaction with sites within the A $\beta$ P channel (Arispe *et al.*, 1993a).

Physiological  $\text{Al}^{3+}$  concentrations ( $\sim 10\text{--}15\ \mu\text{M}$ ) have been reported to be sufficient to block channel conductance even at a driving force of 100 mV. (Arispe *et al.*, 1993a; Pollard *et al.*, 1995). The  $\text{Al}^{3+}$  inhibition of  $\text{A}\beta\text{P}$ -formed channels could be dependent on the type of channel formed. Hirakura *et al.* (1999) reported that  $20\ \mu\text{M}\ \text{Al}^{3+}$  did not block the channels formed by  $\text{A}\beta\text{P}[1\text{--}42]$  in an azolectin bilayer.

**ORGANIC BLOCKERS:** The weak base tromethamine, also known as the buffer Tris, is able to block the  $\text{A}\beta\text{P}[1\text{--}40]$ -formed channel (Arispe *et al.*, 1994). Tromethamine blocks  $\text{A}\beta\text{P}$ -channel current reversibly (millimolar range) (Arispe *et al.*, 1993a). While 1–2 mM tromethamine completely blocked the large channels ( $<400\ \text{pS}$ ), it had very little effect on the giant channels ( $>400\ \text{pS}$ ). It was found that Tris had no effect on  $\text{A}\beta\text{P}[1\text{--}42]$ -formed channel activity at 10 mM concentration (Hirakura *et al.*, 1999). The L-type calcium channel blocking drug nitrendipine is ineffective in blocking  $\text{A}\beta\text{P}[1\text{--}40]$ -formed channels. Similarly, the compound Cognex® (tetrahydroacridine), an inhibitor of acetylcholinesterase, was ineffective (at  $100\ \mu\text{M}$ ) in blocking  $\text{Cs}^{2+}$  permeation of  $\text{A}\beta\text{P}[1\text{--}40]$ -formed channels (Pollard *et al.*, 1995). Congo Red inhibits the ability of  $\text{A}\beta\text{P}[1\text{--}42]$  to form channels. However, once channels are formed, the addition of Congo Red has no effect on these channels. This suggests that aggregates of  $\text{A}\beta\text{P}[1\text{--}42]$  in the aqueous phase are responsible for channel formation, because Congo Red interacts with  $\beta$ -pleated sheets to inhibit aggregation of amyloid-forming peptides (Hirakura *et al.*, 1999).

**Structure–Function Relationship  $\text{A}\beta\text{P}$ -Formed Channels.** It is possible that different  $\text{A}\beta\text{P}$  configurations are involved in the mechanism of action in both endogenous ion transport systems and  $\text{A}\beta\text{P}$ -formed channels. Methods such as circular dichroism spectroscopy and low angle X-ray diffraction have shown the  $\text{A}\beta\text{P}$  protein to have a hydrophobic conformation, with high affinity for lipid environments (Barrow *et al.*, 1992; Hilbich *et al.*, 1991a,b; Jarret and Lansbury, 1992; Mason *et al.*, 1992). Vargas *et al.* (2000) used the displacement current to monitor spontaneous incorporation of  $\text{A}\beta\text{P}[1\text{--}40]$  into bilayers can be detected as an increase in bilayer capacity. Their findings suggest that  $\text{A}\beta\text{P}[1\text{--}40]$  molecules span the bilayer, exposing  $\text{D}_1^-$ ,  $\text{E}_3^-$ ,  $\text{R}_5^+$ ,  $\text{H}_6^+$ ,  $\text{D}_7^-$ ,  $\text{E}_{11}^-$ ,  $\text{H}_{13}^+$ , and  $\text{H}_{14}^+$  to the electric field. They proposed that  $\text{A}\beta\text{P}[1\text{--}40]$  molecules spontaneously acquire  $\beta$ -sheet conformations that allow interactions with anionic phospholipids. Thus,  $\text{A}\beta\text{P}$  molecules are likely to interact with the membrane to form an unregulated  $\text{Ca}^{2+}$ -entry system (Arispe *et al.*, 1993a, 1994, 1996).

In the solid form,  $\text{A}\beta\text{P}$  generally assumes  $\beta$ -sheet structures (Durell *et al.*, 1994). In solution, the protein assumes different proportions of  $\alpha$  helix,  $\beta$  sheet, and random coil and different degrees of aggregation in response to changes in relevant environmental factors, such as ionic strength, pH, and solvent composition and polarity. Durell *et al.* used computer modeling to predict the secondary structure of the membrane-bound protein, and postulate how such a structure may form channels. The 12 N-terminal residues, with alternating polar and hydrophobic residues, were considered to form an  $\alpha$ -hairpin structure with a turn at the  $\text{D}_7^-$ – $\text{S}_8$ – $\text{G}_9$  point. Residues 16–24 were predicted to form an  $\alpha$  helix, because of the general observation that hydrophobic segments form helices in membranes. A turn at residues 25–27 would be followed by the larger C-terminal helix.

It is possible that the A $\beta$ P-formed channel is actually a complex of two or more A $\beta$ P molecules, as synthetic A $\beta$ P has been observed to form stable dimers, trimers, and tetramers in water. The hexamer form has been found to be particularly stable (Durell *et al.*, 1994; Fraser *et al.*, 1992; Inouye *et al.*, 1993). The aggregate view is also supported by the observation that Congo Red, which prevents aggregation of  $\beta$  sheets, also reversibly prevents channel formation by A $\beta$ P[1–42] and A $\beta$ P[25–35] (Hirakura *et al.*, 1999). The rate of aggregation is dependent on A $\beta$ P-amyloid concentration, pH, and temperature. The process of aggregation in vitro has been modelled as A $\beta$ P-amyloid aging (Pike *et al.*, 1991). It is suggested that when A $\beta$ P molecules aggregate in solution, they become neurotoxic (Pike *et al.*, 1991a,b, 1993). This is supported by the observation that monomeric forms lacking  $\beta$ -pleated sheet conformations are relatively inert (Arispe *et al.*, 1994). However, the possibility that fibrils and insoluble deposits may have further effects on neuron structure cannot be ruled out.

Different ion channel models are needed to explain the heterogeneity of A $\beta$ P-formed channels. Durell *et al.* (1994) modelled three types of channel based on the structure of A $\beta$ P, with the limiting premise that channels are composed of aggregates of protein subunits in identical conformations. The first, a “Type I” channel, is formed of four or more protein molecules oriented identically, with their N-terminal  $\beta$  sheets forming the channel. This type of channel can pass ions as large as Cs<sup>+</sup>. At pH of 7 or 7.4, not all Histidine residues would be protonated and the channel would have a net negative charge, which agrees with the observed selectivity of A $\beta$ P for cations. The channel could be parallel, formed of a single layer of parallel subunits, or antiparallel, where two rings come from either side of the membrane, and the hairpin turns interlock in the center. The “Type II” channel is a four-molecule channel composed of a wide “cone” formed by the middle helices and lipid headgroups, and a “spout” composed of the long C-terminal helices. The radius of the pore is around 2.5 Å. The middle helices are at a 45° angle to the membrane plane, leaving four wedge-shaped spaces into which the charged phosphatidylserine lipid headgroups would fit. These negative headgroups would affect the selectivity of the channel. The  $\beta$  sheets would cover the lipid alkyl chains to prevent them being exposed to solvent. The “Type III” channel is formed by a concerted conformational change to the “Type II” channel, in which the C-terminal helices go to a parallel form in the bottom of the membrane, and the middle helices with the lipid headgroups form the transmembrane pore. The minimum radius is 3.0 Å, although smaller sizes could result from adjustments to the peptide–lipid interaction. This conformational change could explain the conductance changes between the <400-pS range and the 400–5,000-pS range (Arispe *et al.*, 1993, 1996; Durell *et al.*, 1994). Such conductance changes could also possibly be explained by a cochannel hypothesis, i.e., incorporation or elimination of A $\beta$ P subunits involved in channel formation (Arispe *et al.*, 1994; Durell *et al.*, 1994). Blockade or partial blockade of large and giant A $\beta$ P[1–40]-formed channels by Zn<sup>2+</sup> suggests that A $\beta$ P molecules in the bilayer can acquire multiple conformations that allow direct interaction between Zn<sup>2+</sup> and some critical domains of the channel (Arispe *et al.*, 1996). The transitions between subconductances occasionally flicker between extremes in the conductance range, suggesting changes in conformation of a single

channel protein rather than the addition or elimination of new protein units (Arispe *et al.*, 1996).

The molecular models also suggest that the A $\beta$ P-formed channel is located asymmetrically within the membrane. This asymmetry is reflected at the putative entrances to the aqueous pore, which has three histidine residues (H<sub>6</sub><sup>-</sup>, H<sub>13</sub><sup>-</sup>, and H<sub>14</sub><sup>-</sup>) and several anionic residues, including D<sub>7</sub><sup>-</sup> and E<sub>11</sub><sup>-</sup>. It is thought that metalloproteases bind Zn<sup>2+</sup> to these sites (Arispe *et al.*, 1996). This is consistent with the primary structure of A $\beta$ P[1–40], which contains two separate local sequences containing histidine (Bush *et al.*, 1994). The local sequence FRHDS contains H<sub>6</sub><sup>-</sup>, while the other local sequence, EVHHQ, contains H<sub>13</sub><sup>-</sup> and H<sub>14</sub><sup>-</sup>. The A $\beta$ P[1–40]-formed channel has rings of H<sub>6</sub>s and D<sub>7</sub>s surrounding one pore entrance. Successive rings of E<sub>11</sub>s, H<sub>13</sub>s, and H<sub>14</sub>s encircle the other entrance (Arispe *et al.*, 1996). This is consistent with the “Type I” channel described here, with the  $\beta$ -sheet turn and C-end at either end of the pore. The molecular configuration in the aggregate form is indicative of a channel with a central pore (Arispe *et al.*, 1994).

#### *Other Channel-Forming APPF Fragments*

It appears that A $\beta$ P fragment is not the only fragment of APP to possess the ability to induce ion channel activity. Fraser *et al.* (1996) examined the possible role of other cleaved products of the  $\beta$ APP to induce ion channel activity. They investigated the ability of various products of  $\beta$ APP to induce membrane ion currents by applying them to *Xenopus oocytes*. Their findings show that the 105-amino-acid C-terminal fragment (CT105) (containing the full sequence A $\beta$ P) is highly neurotoxic and capable of inducing nonselective ion currents when applied from either outside or inside the oocyte. Furthermore, they found that the ion channel activity of CT105 was concentration-dependent and blocked by a monoclonal antibody to A $\beta$ P. A recent study also shows that the C-terminal fragment of  $\beta$ APP forms cation selective channels (Kim *et al.*, 1999b). They used artificial lipid membranes to gain insights into the ionophoric effects of CT105 (10–1,000 nM) as a possible mechanism underlying its cytotoxic effect. The obtained data show that the macroscopic membrane conductance increased with CT105 concentration, as reported previously by Fraser *et al.* (1996). The conductance and kinetic analyses show that the mean unitary conductance of CT105-induced channels was 120 pS and open-state probability was close to 1 at voltages from –80 to +80 mV. In addition, the CT105-induced channels were selective to cations ( $P_K/P_{Cl} = 10.2$ ), being most selective to Ca<sup>2+</sup>. These findings were taken to suggest that CT105 can cause direct neurotoxic effects by forming Ca<sup>2+</sup>-permeable cation channels on neuronal membranes.

## CONCLUSIONS

Genetic mutations or age-related metabolic changes promote neuronal degeneration in AD by increasing production of A $\beta$ P and/or decreasing levels of neuroprotective sAPP alpha. Specific mutations in APP and other alterations of synaptic proteins result in an increased production of A $\beta$ P, which acts as a neurotoxin. The

major events causing Alzheimer's pathology involve A $\beta$ P-induced changes in Ca<sup>2+</sup> homeostasis, generation of ROS, impairment of membrane transport and receptor proteins underlying signal transduction regulating the function of neurons. However, the sequence of these events in AD and other neurodegenerative diseases remains to be clarified. The complexity of determining the sequence of these events is due to the multiplicity of relationships between these events. Do A $\beta$ Ps exert their effects directly (a) by interacting ion transport and receptor proteins, and/or (b) by forming heterogeneous ion channels, and/or indirectly by producing ROS? In addition to the understanding of the nature of A $\beta$ P effects on ion transport systems, it is essential to determine the specific functional sites that confer on A $\beta$ P its cytotoxic activity in order to design a therapeutic block of A $\beta$ P activity early in the course of AD.

### **Endogenous Ion Transport Systems**

The mechanisms and the molecular factors involved in the mechanisms for A $\beta$ P-induced modifications of the various transport pathways are yet to be elucidated: Are the effects of different A $\beta$ Ps on ion channels and ion pumps mediated via a single molecular mechanism? For example, a certain functional site on A $\beta$ P may interact with a certain sequence motif of the ion channel or ion pump. This is important for designing pharmacological treatments. A $\beta$ P-induced enhancements of ion currents, measured in the whole-cell configurations, need to be further verified on the single-channel level to deduce the nature of these effects and to eliminate the possibility that the enhanced current is not due to A $\beta$ P-formed new channels, which may appear to have properties similar to those currents recorded in the whole-cell configurations.

### **A $\beta$ P-Formed Channels**

At the ion channel level, several issues remain to be understood: (a) How are the A $\beta$ P-channels formed? (b) What is the role of different components of the peptide in channel formation and function? (c) How do the channels become heterogeneous and what is the relationship between the various A $\beta$ P configurations and the formed channels? (d) What conformations of A $\beta$ P are pathologic? (e) What are the electrophysiological properties of the pathological A $\beta$ P-formed channels? (f) How do they come to play their role in vivo?

### **Protective Mechanisms**

The fact that neurodegenerative disorders are characterized by damage to selective neuronal populations suggests the need to explore the protective mechanism in the unaffected neuronal populations. It is unlikely that this mechanism is due to the lack of a specific cytotoxic A $\beta$ P conformation in these tissues. Identification of the triggering signal for the abnormal APP activity is needed for an effective therapeutic strategy to prevent AD. The fact that many pathologies mediate their effects via interactions with cell membranes, in a manner similar to those of toxins, suggests that the protein-processing malfunction-induced membrane damage is a central step

in the mechanism of apoptosis. It is unlikely that the age-related malfunction of APP processing is the only protein that undergoes such a process. The evidence, which indicates that in AD several ion transport mechanisms can be affected by soluble, membrane-bound and aggregated forms of A $\beta$ P, suggests the need for a multiple therapeutic strategy to halt the progress and/or reverse AD symptoms.

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