

Detouring destruction: a role for inhibitory neuronal activity in preventing neuronal loss—implications for Alzheimer's disease

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Recent results from the author's efforts and others have indicated approaches that may eventually lead to new therapeutic strategies for combating neurodegenerative effects associated with dementia and Alzheimer's disease (AD). These strategies include unraveling mechanisms that decrease senile plaque accumulation and delay or slow the neurodegenerative progression associated with AD. Recent work addresses whether normally functioning inhibitory brain circuitry can protect and detour neurodegeneration. The aim of these research efforts is that one or a combination of these approaches will develop into an applied therapy that will enhance brain protection mechanisms and add to quality of life for patients with AD.

(Key words: Alzheimer's disease, β -amyloid, benzodiazepine, diazepam, gamma-aminobutyric acid, hippocampus, inhibition, learning, memory, secretase, valium, vaccine)

Efforts are currently under way to develop new strategies that may lead to novel modes of therapy for treating patients with Alzheimer's disease (AD).¹⁻⁴ It is widely held that the condition or set of conditions known as AD is associated with neurodegeneration that results from a variety of sources, including genetic mutations, neurofibrillary tangles, immune responses, neuronal death, and senile plaques.⁵ This neurodegeneration affects many cortical regions of the brain associated with learning and memory, so the cortex continues to be a major target for AD studies.

Before entering into the current strategies under examination, it is noteworthy that one region of cortex, the hippocampus, is a major locus for learning

and memory that accumulates high levels of plaque material in patients with AD. This senile plaque material is primarily composed of an aberrant peptide called β -amyloid that is often associated with neurodegenerative processes pathologically correlated to behavioral symptoms of AD (memory loss). The gene products result because of mutations in genes that affect the processing of amyloid precursor protein (APP). Genetic mutations that are involved in modulating the processing of APP include the presenilins and apolipoprotein E4 (apoE4) genes, which increase the production of one fragment (A β 1-42) and increase the density of plaques and vascular deposits, respectively.

With this framework in place, it follows that therapeutic strategies are targeting mechanisms that decrease plaque material, diminish local immune system influences (see article by Tuppo and Forman in this issue), and maintain neuronal health and functional circuitry. This article will emphasize ongoing approaches that target β -amyloid accumulation or maintain synaptic connec-

tivity of the majority of circuitry in the brain. By contrast, currently available Food and Drug Administration-approved drugs for AD target only one mechanism (acetylcholine esterase inhibition) intended to preserve cholinergic synaptic connections. This article builds on the rationale underlying the current indications to include neuroprotection of the two major neurotransmitter systems in the central nervous system (CNS)—glutamate and gamma-aminobutyric acid (GABA). The remainder of this article focuses on these strategies by first addressing mechanisms that decrease β -amyloid accumulation then focusing on work that targets neuronal mechanisms and optimal neuronal functioning of circuitry.

Can the enzyme responsible for producing the β -amyloid fragment be inhibited?

A strategy currently being intensively examined is an effort to inhibit enzymes that result in the production of the β -amyloid fragments that become the primary constituent of developing plaque accumulation in the brain. By targeting enzymes responsible for producing "aberrant" β -amyloid peptide, the amount of plaque that forms would be substantially diminished, hence reducing plaque formation in the brain. Enzymatic cleavage results from the gamma secretase activity. Neurons, astrocytes, and microglia of the brain produce the β -amyloid, which in turn is excreted and becomes available to accumulate in senile plaques. Several articles have reviewed this progress in more detail.^{6,7}

An analogous approach with similar goals involves the development of an AD vaccine.⁸⁻¹⁰ The mechanism underlying this approach is less well characterized, but the result is similar in that vaccine administration has led to a reduction in plaque accumulation in transgenic mice engineered to produce plaque load by overexpressing β -amyloid.⁸ Thus, both approaches implicate β -amyloid as the culprit and attempt to reduce the amount of β -amyloid in the brain. Despite these innovations, genetic mutations resulting in production of β -amyloid occur only in a small number of

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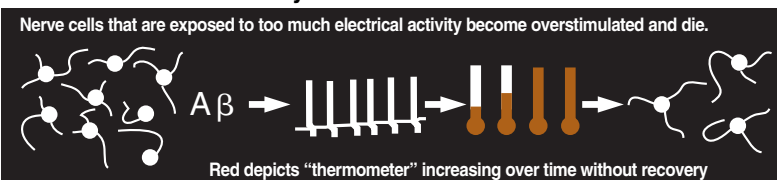
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Figure 1.

Neurodegeneration

When electrical activity increases (depicted by white impulses), it causes overstimulation (depicted by thermometers that do not recover) leading to an increase in nerve cell death. Note how the number of nerve cells decreases from the left picture to the right.

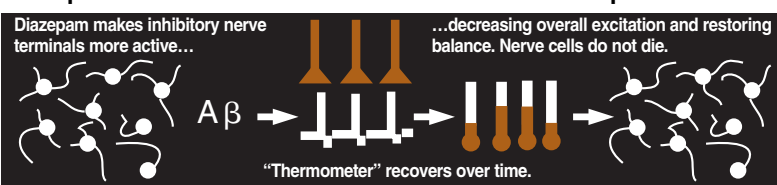
Too much electrical activity kills nerve cells.



Restoring balance

Diazepam protects nerve cells from β -amyloid effects by decreasing overall electrical activity. Overstimulation is prevented (depicted by the recovery of the "mercury" in the thermometers over time.) Note that the number of nerve cells stays constant, left to right.

Diazepam decreases overstimulation so nerve cells are protected.



patients with AD. Yet it is instructive to determine if reducing plaque load will not only result in improved behavioral symptoms but also preclude the condition entirely. Recently, it was reported that vaccine treatment improves behavioral tasks in studies performed on mice.⁹

How does plaque material lead to neuronal loss?

Many laboratories have shown that overstimulation of excitatory brain cell activity (excitotoxicity) by introducing the excitatory neurotransmitter glutamate and/or β -amyloid peptides leads to neuronal destruction.¹¹⁻¹⁴ Moreover, overstimulation can be initiated by plaque

material or other stimuli, and the resulting neuronal loss from exposure to β -amyloid is termed the *β -amyloid-induced excitotoxicity theory* (Figure 1, top). This theory claims that overstimulated neurons are not able to recover their cytosolic free calcium loads after an activity-induced increase in calcium. Calcium is thought to rise to high levels that cannot recover owing to loss of calcium regulatory homeostasis (see Figure 1, top). The higher level of calcium ($>1 \mu\text{M}$) is thought to activate remodeling enzymes (calpains) in an unregulated manner that can lead to the destruction of the neuronal plasma membrane and hence cell death.¹³ In addition to loss

of calcium homeostasis, the downstream production of free radicals is also thought to enhance the neurodegenerative process.¹³

Despite the source of the overstimulation and its downstream effects, excitotoxicity could not occur without functioning neuronal circuitry in the brain, so the synaptic connections are indispensable components for normal communication, including memory storage. A component of the memory loss symptom of AD surely includes this neuronal loss, which simultaneously adds to the neurodegenerative debris while destroying synaptic connections thought to be integral to memory retention.

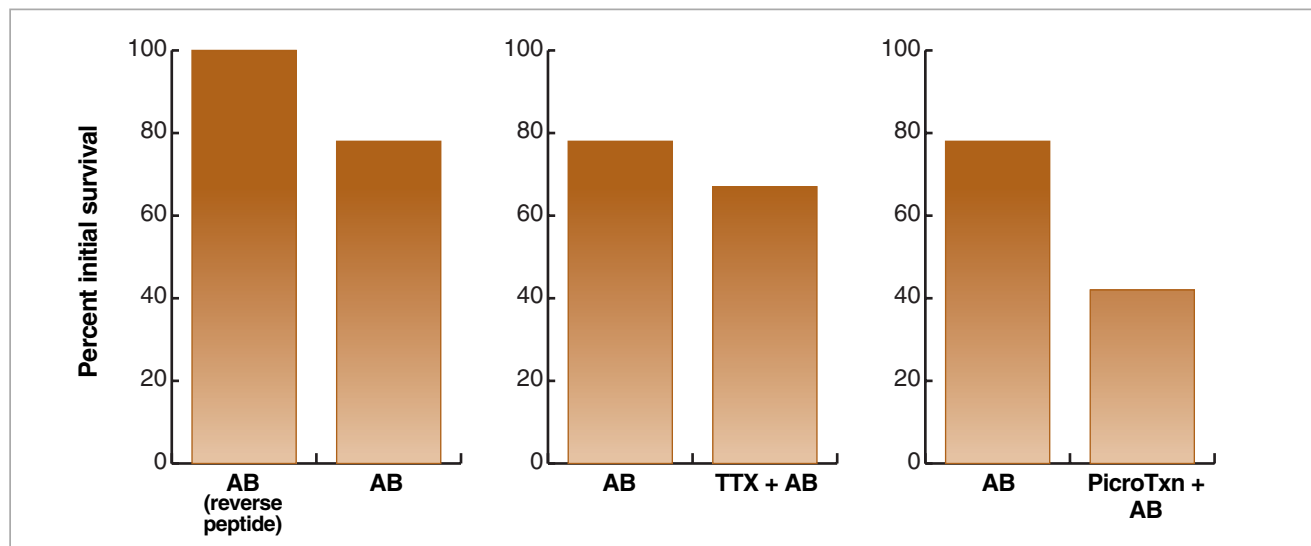


Figure 2. Pharmacologically blocking GABA_A receptors with picrotoxin decreases neuronal survival levels below that of β -amyloid alone. (A) Control: β -amyloid kills cells more than reverse peptide. (B) β -Amyloid plus TTX kills cells as much if not more than β -amyloid alone. (C) Picrotoxin plus β -amyloid exacerbates cell death more than β -amyloid alone. Modified and reproduced by permission from Brain Research.

Inhibitory activity inherent to normal brain function may protect neurons from dying

Another current strategy that does not depend on targeting the source of the neuronal imbalance instead targets an inherent source of protection and fosters neuronal protection by reducing the intensity and volume of overstimulation using pharmacologic approaches. Recall that the major excitatory neurotransmitter in the CNS is glutamate and the major inhibitory counterpart in synapses is GABA. If neurodegeneration is caused by overstimulation, perhaps overstimulation can be counteracted by enhancing inhibitory GABAergic synaptic activity in the brain. The role that the inhibitory component may be playing during disease states such as AD has only recently been addressed.¹⁵⁻¹⁹ Because of this, progress in this emerging field will focus on recent efforts only.

Pharmacologically teasing out the role of inhibitory activity in hippocampal circuits exposed to β -amyloid

To look at the effects of excitation and inhibition on β -amyloid-induced cell death, it is important to ensure that excitation or inhibition is uniformly stimulated in the circuits of neurons. For this reason, primary cell culture of hippocampal neurons is often used because these cells form circuits in culture and are uniformly exposed to treatments. Studies in our laboratory and others have used this approach to test effects of stimulation on cell death in the cultures by analyzing the percentage of cells that survive after using pharmacologic agents to stimulate the circuits. Previous studies demonstrated that the excitatory neurotransmitter glutamate in the presence of β -amyloid leads to cell death.¹¹⁻¹³ Thus, it follows that blocking all neuronal activity in the presence of β -amyloid would protect the neurons from death.

Interestingly, recent evidence demonstrated that tetrodotoxin (TTX), a sodium channel blocker that blocks all neuronal activity in the circuit, resulted in death of circuits exposed to β -amyloid peptides (*Figure 2*). Thus, those observations were contrary to the prediction from a β -amyloid-induced excitotoxic-

ity perspective alone. In fact, TTX in the presence of β -amyloid led to an increase in cell death (decreases survival; *Figure 2*) compared with when neurons were treated with TTX or β -amyloid alone.¹⁵ Therefore, the contribution of the excitatory circuitry alone could not account for the extent of cell death observed.

Mechanism underlying increased cell death is inherent to the circuit

Could it be that the inhibitory component, which normally was active in the circuitry, was also being inhibited by the TTX? To further test this, a more specific inhibitory circuitry blocker, picrotoxin (or bicuculline), was added. In this case, the prediction would be that an inhibitory-activity blocker would only block the GABAergic synaptic contribution, thus allowing all excitatory activity to continue uninhibited. Under these circumstances, it might be expected that neuronal death would increase, even more than in the presence of β -amyloid alone. In fact, neuronal activity was not protected and neuronal survival was decreased¹⁵ (*Figure 2*). These predictions were supported by neuronal survival counts and suggest that an inhibitory component is normally involved in protecting excitatory circuitry from being overstimulated because of β -amyloid.

Thus, when this inhibitory component was blocked, the contribution of GABAergic inhibitory impulses no longer normalized the excitatory electrical activity that was killing the neurons (*Figure 2*).

Can stimulation of inhibitory synapses protect neurons from death?

If turning inhibitory circuitry input off leads to cell death, perhaps enhancing the inhibitory activity could improve the situation. With this in mind, experiments were designed to test whether a benzodiazepine class sedative, such as diazepam (Valium, which functions mechanistically as a GABA_A agonist receptor), could enrich inhibition and protect neurons from death due to β -amyloid. Further, because benzodiazepines are currently prescribed to patients with AD for agitation, could it be that this drug might also be able to reduce the amount of neurodegenera-

tion in circuits of hippocampal brain neurons? Because it is widely thought that memories are stored in arrays of synapses, perhaps preserving those synapses pharmacologically by applying a low-level benzodiazepine might detour destruction due to overstimulation and preserve the neuronal circuitry.

For more background on neuroprotection mechanisms, which is beyond the scope of this article, the reader is referred to some recent reviews providing more information on the approaches introduced here.²⁰⁻²²

A role for benzodiazepines to protect neurons from β -amyloid-induced damage?

Preliminary data support the hypothesis that the benzodiazepine diazepam provides protection from death to neurons exposed to β -amyloid peptides.²¹ Neurons exposed to β -amyloid in the presence of low levels of benzodiazepines are protected to similar levels as those that are untreated or treated with reverse peptide (*Figure 1*; bottom). Moreover, a behavioral study recently appeared claiming that cognitive abilities are compromised in patients with AD who received the benzodiazepine antagonist flumazenil (Romazicon).²² Physiologic studies in transgenic mice overexpressing presenilin-1 appear to indicate a role for benzodiazepines in normalizing synaptic potentiation.²³

Taken together, these results suggest both on a behavioral and mechanistic level that benzodiazepines may protect neurons and temper synaptic potentiation, whereas pharmacologic removal of that protection with flumazenil appears to be observable on the behavioral level with worsening symptoms of the disease.

Comments

Whether these recent scientific efforts in the laboratory will translate into a therapeutic indication for benzodiazepines for this purpose remains to be seen. In the meantime, it is encouraging that research is gaining momentum by further examining prospective modes of therapy aimed at decreasing plaque accumula-

tion and neuronal death due to insults such as β -amyloid deposition in the brain.

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