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Biochemical, Physiological, and Behavioral Characterizations of the Cholinergic Basal Forebrain Lesion Produced by 192 IgG-Saporin

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BACKGROUND

The Cholinergic Hypothesis

Selective lesioning of the cholinergic neurons in the basal forebrain nuclei was a highly sought goal for use as an animal model of Alzheimer's disease. Autopsy studies of Alzheimer's-diseased brain tissue found that a substantial loss of the cholinergic innervation of the cerebral cortex and hippocampus was a prominent feature of this disease, and the degree of this neuron loss was highly correlated with the degree of dementia *(1,2)*. Subsequent research confirmed that cholinergic neurons originating in the nucleus basalis of Meynert, the diagonal band of Broca, and the medial septal nucleus, which terminate mainly in the cortex, olfactory bulb, and hippocampus *(3,4)*, are destroyed in the progression of Alzheimer's disease. This loss occurred earlier than the degeneration of other types of neurons, and the scale of this cholinergic cell death was massive. The similarity to dopaminergic depletion in Parkinson's disease was evident, and the cholinergic hypothesis for the dementia of Alzheimer's disease was proposed. The need for an animal model to test therapeutic strategies fueled basic research about the function of this cholinergic basal forebrain (CBF) system.

Unselective CBF Lesions

Lesioning methods already in use on rat brains included mechanical lesions such as fimbria/fornix transections to remove cholinergic (and other)

From: *Molecular Neurosurgery With Targeted Toxins*
Edited by: R. G. Wiley and D. A. Lappi © Humana Press Inc., Totowa, NJ

input to the hippocampus and radio-frequency or electrolytic lesioning of discrete regions. Excitotoxins, many of which are potent agonists at glutamate receptors that destroy cells bearing these receptors by excessive excitation or calcium ion overload, were a considerable improvement over mechanical lesions because perikarya, but not fibers en passage, are destroyed *(5,6)*. Unfortunately, many different kinds of cells (including glia) bear glutamate receptors and, although cholinergic neurons are susceptible to these toxins as demonstrated by depletion of cholinergic markers, histological examination has demonstrated that other cell types, especially GABAergic (γ-aminobutyric acid) neurons, are destroyed when excitotoxins are infused into the nucleus basalis magnocellularis of rats *(7–9)*.

The ethylcholine aziridinium ion AF64A was developed in a well-reasoned attempt to produce greater specificity for cholinergic neuron destruction. It targeted a specific membrane protein present on cholinergic neurons, the high-affinity choline transporter *(10)*. Unfortunately, the specificity of this toxin for cholinergic neurons in vivo was difficult to ensure because of the high alkylating reactivity of the aziridinium ion. When injected intracerebroventricularly (icv), it can destroy cholinergic innervation of the hippocampus, but its utility as a specific cholinotoxin is less than was originally hoped *(11,12)*.

Development of 192 Immunoglobulin G-Saporin

Ultimately, the goal of producing a selective lesion of CBF neurons was achieved by the collaboration between groups led by Dr. Douglas Lappi and Dr. Ronald Wiley and the coupling of two compounds, saporin and an antibody against the rat low-affinity (p75) nerve growth factor receptor (NGF-R). This immunotoxin, 192 immunoglobulin G-saporin (IgG-sap), built on the idea of coupling a compound selective for cholinergic neurons with a toxin. Saporin is cytotoxic only when it gains access to the interior of cells and inhibits protein synthesis. The immunotoxic compound relies on internalization of the antibody for the desired selectivity. Such chimeric suicide transport agents were under investigation for targeting malignant cells, as well as other uses for selective cell destruction in a living organism. Of the toxins tested, saporin proved to have the greatest efficacy in cell destruction when coupled to a molecule that conferred specificity and applied to neurons in the central nervous system *(13–17)*.

A monoclonal antibody to the rat p75 nerve growth factor-receptor (NGF-R), 192 IgG is endocytosed on binding to the receptor and is retrogradely transported from axon terminals to cell bodies *(18)*. When radioiodinated and injected icv, it was found to accumulate preferentially in the basal fore-