LINOPIRDINE
A Depolarization-Activated Releaser of Transmitters for Treatment of Dementia

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INTRODUCTION

Advances in medicine are giving rise to extended life expectancy and thus diseases which usually affect the elderly population will increase in prevalence. Alzheimer’s disease (AD), the leading cause of dementia in the aged population, is devastating to the patients as well as their family members. This is a costly disease to the individuals and to society. Although an enormous effort is being made to understand the cause of this disease and to develop therapies for it, AD remains one of the foremost challenges to medical research today.

The dementia occurring in AD is associated with dysfunction and death of neurons in a variety of cell populations, including cholinergic, monoaminergic, and peptidergic systems\textsuperscript{1-3}. A substantial amount of data support a central role of acetylcholine (ACh) in the cognitive dysfunction seen in AD and aging. Cerebral cortical ACh synthesis declines as a function of age in animals\textsuperscript{4,5}. One of the earliest observed neurochemical changes in AD is the profound loss of neocortical cholinergic innervation.\textsuperscript{6-9} This loss has been found to be correlated with the degree of dementia found in the disease. Lesioning of cholinergic cell bodies in the nucleus basalis magnocellularis projecting to the neocortex induces marked deficits in cognitive performance in experimental animals\textsuperscript{3,10,11} and these cognitive deficits can be attenuated by cholinergic agonists, ACh releasing agents, and acetylcholinesterase inhibitors.\textsuperscript{2,12} These observations have led to what has been called the \textit{cholinergic hypothesis} of AD which suggests that the cholinergic losses observed in AD lead directly to the cognitive and mnemonic deficits observed in the disease. However, with the wide range of neurochemical alterations now documented in AD\textsuperscript{13-15} the \textit{cholinergic hypothesis} appears to be an oversimplification.

Strategies to enhance cholinergic function in brain (e.g. precursor loading, acetylcholinesterase inhibitors, and cholinergic receptor agonists) represent the predominant approaches that are currently being clinically evaluated to reverse some of the cognitive deficits seen in AD (for a review see Davis et al.\textsuperscript{16}). Precursor loading with choline or phosphatidylcholine failed to have a significant effect on AD symptoms.\textsuperscript{17,18} Treatment with acetylcholinesterase inhibitors such as physostigmine\textsuperscript{19,20} and tetrahydroaminoacidine\textsuperscript{21,22} to retard the degrada-
tion of ACh has had some limited benefit, but side effects have also been noted. Initial attempts to treat AD with direct cholinergic agonists were limited by low efficacy and side effect issues. However, the identification of multiple subtypes of muscarinic receptors has stimulated interests in developing M₁ receptor agonists to treat AD. Finally, an alternative approach to treat AD is to stimulate cholinergic function by enhancing the release of ACh.

A number of compounds, such as the aminopyridines, increase both basal (release in the absence of a stimulus) and stimulus-evoked release of ACh. This type of non-specific activation may lead to untoward events such as neurotransmitter depletion, overload toxicity, and desensitization. Therefore a compound which specifically enhances evoked release and not basal release should enhance normal synaptic activity and increase the signal-to-noise ratio during neuronal transmission. During the evaluation of a number of compounds that had the potential to reduce cholinergic system dysfunction and enhance cognitive function by increasing ACh levels in the brain, linopirdine (DuP 996; AVIVA®) 3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one) was identified. Linopirdine represents a novel class of compounds which are depolarization-activated releasers of neurotransmitters. This chapter describes the preclinical results of linopirdine.

**SYNTHESIS**

Linopirdine was synthesized according to the method shown in Figure 1. Diphenylamine was added to a solution of oxalyl chloride and distilled and refluxed to form indoline-2, 2-dione. 4-Picoline in acetic acid was then added, followed by acetic anhydride, and then water and isopropanol. The mixture was cooled and filtered to yield 3-(4-pyridinylmethylidene)-1-phenylindolin-2-one (I). Sodium borohydride pellets were added to a slurry of I in methanol and then 10 N sodium hydroxide was added. A solution of 4-picolychloride HCl was added to this mixture and followed by 10 N sodium hydroxide. The solids were collected by filtration, washed with water, and dried in a vacuum. The resultant crude product was recrystallized in isopropanol and water to yield the product.

**NEUROTRANSMITTER RELEASE ENHANCEMENT**

Linopirdine enhancement of K⁺-stimulated [³H]ACh release was demonstrated using a two pulse K⁺-depolarization paradigm in superfused rat brain slices preloaded with [³H]choline. The effect of 10⁻⁵ M linopirdine on the release of ACh in vitro is shown in Figure 2. Addition of linopirdine to brain slices has no effect on the basal release of ACh. The ACh release enhancing effect of linopirdine is dose dependent (Figure 3). A significant effect on enhancement of ACh release can be observed at 10⁻⁶ M and reaches maximum at 10⁻⁵ M. Similar effects of linopirdine are observed using slices of rat cerebral cortex, hippocampus, and striatum. The release enhancing effects of linopirdine is not limited to cholinergic processes alone. The release of tritium from brain slices preloaded with [³H]dopamine (DA), [³H]serotonin (5-HT), [³H]glutamate (Glu) and [³H]d-aspartate, as well as [³H]choline, is enhanced by linopirdine. Linopirdine enhances the release of the newly synthesized pool of radio-labeled neurotransmitters as well as enhances the release of both endogenous ACh from neocortical slices and DA from striatal slices. Since the neuronal deterioration which is involved in AD involves axons and terminals emanating from multiple neuronal cell types, the ability of linopirdine to enhance the release of multiple neurotransmitters offers an advantage over current AD therapies which are aimed at stimulating the cholinergic system alone.