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Donepezil markedly potentiates memantine neurotoxicity in the adult rat brain

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Abstract

The NMDA antagonist, memantine (Namenda), and the cholinesterase inhibitor, donepezil (Aricept), are currently being used widely, either individually or in combination, for treatment of Alzheimer's disease (AD). NMDA antagonists have both neuroprotective and neurotoxic properties; the latter is augmented by drugs, such as pilocarpine, that increase cholinergic activity. Whether donepezil, by increasing cholinergic activity, might augment memantine's neurotoxic potential has not been investigated. In the present study, we determined that a dose of memantine (20 mg/kg, i.p.), considered to be in the therapeutic (neuroprotective) range for rats, causes a mild neurotoxic reaction in the adult rat brain. Co-administration of memantine (20 or 30 mg/kg) with donepezil (2.5–10 mg/kg) markedly potentiated this neurotoxic reaction, causing neuronal injury at lower doses of memantine, and causing the toxic reaction to become disseminated and lethal to neurons throughout many brain regions. These findings raise questions about using this drug combination in AD, especially in the absence of evidence that the combination is beneficial, or that either drug arrests or reverses the disease process.

Keywords: Memantine; Donepezil; Tacrine; Neurotoxicity; Vacuoles; NMDA antagonist; Cholinergic; Glutamatergic; Cell killing; Excitotoxic; Dendrotoxic

1. Introduction

In acute brain injury conditions, such as hypoxia/ischemia and head trauma, glutamate accumulates in excitotoxic concentrations at NMDA receptors, and blocking these receptors can be neuroprotective (Choi, 1992; Lipton and Rosenberg, 1994; Olney and Farber, 1995; Rothman and Olney, 1987). Reasoning from this line of evidence, it has been hypothesized that glutamate, by chronic low-grade over-stimulation of NMDA receptors, may contribute to the neuropathology of Alzheimer's disease (AD), and that drugs that block NMDA receptors might be neuroprotective in AD (Danysz and Parsons, 2003).

In addition to its direct excitatory actions in the brain, there is evidence that glutamate is a major regulator of inhibitory tone (Olney, 1994, 1995; Olney et al., 1991, 1997). By tonically stimulating NMDA receptors on GABAergic inhibitory neurons that modulate both glutamatergic and cholinergic excitatory pathways, glutamate exerts an inhibitory restraining influence over these excitatory pathways. Blocking NMDA receptors in this circuitry abolishes GABA's inhibitory action, thereby disinhibiting the glutamatergic and cholinergic excitatory pathways and causing excessive excitatory (excitotoxic) activity that injures or kills neurons that they innervate (Olney, 1994; Olney et al., 1989, 1991). Thus, any condition leading to impairment or hypofunction of NMDA receptors might be conducive to disinhibition of these excitatory pathways. There is considerable evidence in several animal species (Gonzales et al., 1991; Magnusson and Cotman, 1993; Tamaru et al., 1991; Wenk et al., 1991) that the NMDA transmitter system becomes progressively less functional with increasing age, and it has been reported (Ulas and Cotman, 1997) that NMDA receptor hypofunction is even more extreme in the brains of AD patients than in age-matched controls. Based on these and related lines of evidence, NMDA receptor hypofunction and related disinhibition of excitatory pathways has been postu-

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lated as a disease mechanism that might contribute to the neuropathology of AD (Olney and Farber, 1995; Olney et al., 1997). According to this proposal, it would be hypofunction of NMDA receptors that generates conditions leading to excitotoxic neuropathology in AD, whereas the competing hypothesis mentioned above postulates over-stimulation (hyperfunction) of NMDA receptors leading to excitotoxic neuropathology in AD. It is important to recognize that these are conceptually opposite proposals, because for therapeutic intervention in AD one hypothesis calls for decreased NMDA receptor activity, and the other hypothesis calls for increased receptor activity.

The hope that NMDA antagonist drugs might be used therapeutically to prevent excitotoxic neurodegeneration has been nurtured for nearly two decades, but it has been difficult to bring the concept to fruition, the most significant problem being that at doses that exert neuroprotective effects, NMDA antagonists cause adverse side effects ranging from memory dysfunction and psychotic reactions in humans (Grotta et al., 1995; Herrling, 1994; Krystal et al., 1994) to acute injury and/or death of neurons in animal brain (Allen and Iversen, 1990; Fix et al., 1993; Hargreaves et al., 1993; Olney et al., 1989, 1991; Wozniak et al., 1999). Memory dysfunction arises at least in part from a direct interference in the memory functions sub-served by NMDA receptors (Kawabe et al., 1998; Meehan, 1996; Morris et al., 1986; Nguyen and Kandel, 1996; Wozniak et al., 1990), but the disinhibition mechanism mentioned above is thought to be responsible for the other side effects.

An important feature of the disinhibition-mediated neurotoxic syndrome is that it involves excessive release of both glutamate and acetylcholine, which are the two main excitatory transmitter systems in the brain. NMDA receptor blockade leading to suppressed GABAergic inhibition and excess excitation of both glutamate (non-NMDA) receptors and cholinergic muscarinic receptors constitutes a latent pathological state of increased excitatory tone. Any additional destabilizing influence favoring increased excitation can have serious neurotoxic consequences. Accordingly, it has been found that drugs such as pilocarpine that increase cholinergic activity, markedly potentiate the neurotoxicity of NMDA antagonist drugs (Corso et al., 1997; Wozniak et al., 1999). The potentiated neurotoxic syndrome is mediated by excessive release of glutamate at non-NMDA receptors, excessive release of acetylcholine at muscarinic receptors, and additional increased stimulation of muscarinic receptors by pilocarpine. The excitotoxic neuronal injury induced by treatment with an NMDA antagonist alone is potentially reversible and regionally confined, or may be irreversible and disseminated, depending on the duration of NMDA receptor blockade (Olney et al., 1991; Fix et al., 1993). Drugs that inhibit muscarinic cholinergic activity prevent NMDA antagonist neurotoxicity (Olney et al., 1991), whereas drugs that increase cholinergic activity cause the neurotoxic reaction to become more widespread and more lethal to neurons, and they cause the neurotoxic manifestations to be triggered by low doses of the NMDA antagonist that would not by themselves be neurotoxic (Corso et al., 1997; Wozniak et al., 1999).

Proponents of the hypothesis that a chronic low-grade over-stimulation of NMDA receptors may underlie the neurodegenerative process in AD have developed a specific NMDA antagonist drug, memantine, as an anti-excitotoxic neuroprotective therapy for AD patients (Danysz and Parsons, 2003; Parsons et al., 1999). They have described memantine as a unique NMDA antagonist with special receptor binding kinetics that allow it to block NMDA receptors without neurotoxic consequences (Chen et al., 1998; Danysz and Parsons, 2003; Parsons et al., 1999). Memantine has been evaluated in human clinical trials and reportedly is beneficial for AD patients at doses that are free from neurotoxic side effects (Reisberg et al., 2003; Tariot et al., 2004). Prior to the introduction of memantine, cholinesterase inhibitors were the only drugs approved for treatment of AD patients and they were approved specifically for patients with mild to moderate AD. When memantine was approved by the FDA as a treatment for patients with moderate to severe AD, no precautions were stipulated regarding a potential adverse interaction between memantine and cholinesterase inhibitors. In fact, some of the research intended to establish the safety and efficacy of memantine in AD involved administration of memantine to patients already receiving cholinesterase inhibitor therapy (Hartmann and Mobius, 2003; Tariot et al., 2004). While no rigorous data are available, the clinical reality is that many AD patients, regardless of the stage of illness, are currently receiving combined treatment with memantine and a cholinesterase inhibitor.

As mentioned above, there is ample evidence from animal studies that combined administration of an NMDA antagonist with drugs that increase cholinergic activity can have serious neurotoxic consequences. Cholinesterase inhibitors non-specifically increase cholinergic activity by prolonging the action of acetylcholine at all of its receptors. We searched for, and could not find any published toxicological studies appropriately designed to evaluate the safety of memantine/cholinesterase inhibitor drug combinations and, therefore, undertook the present study in which adult rats were treated with memantine alone, or together with tacrine or donepezil, and the brains were examined 2–48 h later for evidence of either acute and potentially reversible injury or irreversible neurodegeneration.

2. Methods

2.1. Animals and drugs

Harlan Sprague–Dawley female retired breeders (6–8 months old) were used because sensitivity to the neurotoxic effects of NMDA antagonists is influenced both by age and gender—female and fully adult rats are more sensitive than male or immature rats (Farber et al., 1995; Fix et al., 1995;