Neuroscience 164 (2009) 941–947

BLOCKADE OF GABA(B) RECEPTORS COMPLETELY REVERSES AGE-RELATED LEARNING IMPAIRMENT

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Abstract—Impaired cognitive functions are well-described in the aging process. GABA(B) antagonists can facilitate learning and memory in young subjects, but these agents have not been well-characterized in aging. Here we show a complete reversal of olfactory discrimination learning deficits in cognitively-impaired aged Fischer 344 rats using the GABA(B) antagonist CGP55845, such that drug treatment restored performance to that on par with young and cognitively-unimpaired aged subjects. There was no evidence that this improved learning was due to enhanced olfactory detection abilities produced by the drug. These results highlight the potential of targeting GABA(B) receptors to ameliorate age-related cognitive deficits and demonstrate the utility of olfactory discrimination learning as a preclinical model for testing novel therapies to improve cognitive functions in aging.

Key words: CGP55845, aging, GABA(B) receptor, cognitive enhancement, odor, learning.

The number of people over 65 living in the US is expected to increase from 35 to 72 million by 2030, comprising almost 20% of the US population (US Census Bureau, 2004). Further estimates suggest that as many as 30% of these individuals will develop cognitive decline ranging from severe dementia associated with pathological conditions such as Alzheimer's disease (8–9%) (Freedman et al., 2002, Centers for Disease Control and Prevention and The Merck Company Foundation, 2007), to mild cognitive impairment (MCI, 20%) (Lopez et al., 2003; Morris, 2005). Cognitive disabilities associated with aging create a significant burden for individuals and family members as well as a financial strain on the healthcare system. As such, there is significant interest in identifying therapies to slow and/or counteract loss of learning and memory capacities associated with the aging process.

Basal forebrain cholinergic and GABAergic projection neurons are well positioned to directly impact mnemonic function in hippocampus and other medial temporal lobe structures, the functions of which are sensitive to precipitous decline in aging (e.g., explicit/declarative and spatial learning and memory; Frotscher and Leranth, 1986; Freund and Antal, 1988; Baxter et al., 1996; Pang and Nocera, 1999). As such, these transmitter systems are logical targets for therapies to improve cognitive capacities in aging. Indeed, drugs that enhance cholinergic activity (either through direct agonistic actions at cholinergic receptors or by increasing ACh availability by inhibiting acetylcholinesterase (AChE) activity) enhance a variety of cognitive functions across species (for review, please see Parent and Baxter, 2004) and most currently available treatments for age-related cognitive decline are AChE inhibitors (Fischer et al., 1989; Smith and Booze, 1995; Gibbs, 1998; Gilmor et al., 1999; Doggrell and Evans, 2003; Jones, 2003; Parent and Baxter, 2004). Indeed, MCI patients taking AChE inhibitors show increased hippocampal activity and improved performance on explicit memory tasks, demonstrating that these drugs do offer clinical benefit (e.g., Gron et al., 2006). However, this therapeutic avenue in isolation has limitations, as the cognitive enhancing effects of AChE inhibitors in aged individuals are transient; after 3 years, MCI patients with and without AChE inhibitor treatment are cognitively equivalent (Petersen et al., 2005). Moreover, this class of drugs only appears effective in improving mild loss of cognitive functions and offers little benefit to aged individuals with moderate to severe learning and memory deficits (Kaduszkiewicz et al., 2005; Birks and Flicker, 2006; Pelosi et al., 2006; Raschetti et al., 2007).

Given that data from lesion studies in young subjects indicate that coordinated actions of cholinergic and GABAergic signaling are critical to many aspects of cognition affected by age (Baxter et al., 1995; Pang and Nocera, 1999; Pang et al., 2001; Parent and Baxter, 2004; Yoder and Pang, 2005), drug therapies targeting the GABAergic system may offer novel but complementary treatment avenues for dementia. Specifically, antagonists at the GABA(B) receptor appear to be promising candidates, as compounds from this drug class reportedly enhance cognitive function across a wide range of tasks and species in young subjects (Mondadori et al., 1996a,b; Flood, 1998; Getova and Bowery, 1998; Escher, 2004; Froestl et al., 2004; Helm et al., 2005; Berta et al., 2009). For example, in young rodents and non-human primates, the most well-studied GABA(B) receptor antagonist, SGS742 (CGP36742), improves performance in a two-way active avoidance task and spatial reference memory in the eight-arm radial and Morris water mazes (Getova and Bowery, 2001; Froestl et al., 2002; Helm et al., 2005; Chan et al., 2006). In the Helm et al. (2005) study, improved memory was associated with decreased hippocampal CREB2 (ATF4) activity, indicating one site of action and possible mechanism following systemic administration of

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Abbreviations: AChE, acetylcholinesterase; MCI, mild cognitive impairments; SD, standard deviation.

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doi:10.1016/j.neuroscience.2009.08.055

this compound (Vernon et al., 2001; Chen et al., 2003; Helm et al., 2005). The clinical utility of this class of pharmaceuticals is further supported by the wide range of effective doses at which enhanced learning and memory is observed in young subjects and few side effects associated with the efficacious doses (Blake et al., 1993; Mondadori et al., 1996a,b; Getova and Bowery, 2001; Helm et al., 2005; Chan et al., 2006; Emson et al., 2007). Nevertheless, surprisingly few studies have examined GABA(B) antagonists as a possible treatment for age-related cognitive deficits. This was the goal of the current report.

In this study, the GABA(B) antagonist CGP55845, a compound that to date has not been investigated within the context of aging, was assessed for its ability to improve odor discrimination learning deficits in a subset of aged F344 rats. In humans, olfactory functions are increasingly being recognized as vulnerable to age, and olfactory identification and discrimination deficits have been linked to other types of more troublesome learning and memory dysfunction such as declarative memory processes mediated by the medial temporal lobe (Gabrieli, 1996; Freedman et al., 2002; Eibenstein et al., 2005; Wilson et al., 2006). In agreement with these results, our group recently reported such a relationship in aged F344 rats (LaSarge et al., 2007). As observed among humans, considerable variability naturally occurs among the aged F344 rat population such that some aged rats maintain cognitive abilities on par with young cohorts while others develop marked and significant cognitive impairment with advancing age (Bizon et al., 2009). We observed that the same subpopulation of aged F344 rats that demonstrates impaired spatial reference memory is also impaired in the ability to discriminate odors despite the fact that these rats have comparable odor detection abilities and can discriminate other sensory stimuli as well as young and aged-unimpaired cohorts (LaSarge et al., 2007).

Notably, in our previous study, odor discrimination learning abilities in individual aged F344 rats were highly consistent across novel odor discrimination pairs. Aged rats classified as “learning-impaired” eventually reached criterion levels of performance on a given discrimination problem, but there appeared to be neither savings of prior learning rules nor a practice effect across subsequently presented odor pairs. Indeed, these rats were just as impaired on their third odor discrimination problem as on their first. As such, the olfactory discrimination task presented itself as particularly well-suited for assessing the ability of pharmacological agents to improve age-related cognitive impairment. First, the task is as effective as water maze for identifying learning-impaired rats within the aged F344 study population. Second, the reliability of the olfactory discrimination learning deficit in aged rats allows for the use of a within-subject experimental design in which performance of each subject can be evaluated with and without drug treatment. Using this design, we report here that acute administration of the GABA(B) antagonist CGP55845 completely reverses olfactory discrimination learning deficits in aged learning-impaired rats, returning performance to that on par with young subjects.

EXPERIMENTAL PROCEDURES

Young adult (6 months, $n=10$) and aged (22 months, $n=17$) male F344 rats obtained from the National Institute on Aging colony (Harlan, IN, USA) were individually housed in the AALAC-accredited Psychology Department vivarium at Texas A&M University with a regular 12-h light/dark cycle (lights on 8:00) and climate control at 25 °C. Rats were given free access to food and water except during discrimination testing, when they were food-restricted to 80% of their free-feeding weights. All rats were screened daily for health problems including but not limited to cataracts, jaundice, food and water intake, and the appearance of tumors. Sentinel rats housed in the same room were further screened for a range of pathogens, and all blood work was negative throughout testing. All animal procedures were conducted in accordance with approved institutional animal care procedures and NIH guidelines. We made every effort to minimize the number of animals used and their suffering.

Olfactory discrimination learning was tested according to LaSarge et al. (2007). Briefly, the test apparatus consisted of an opaque plastic box ($49\times33\times28$ cm^3) divided by an opaque Plexiglas barrier into holding (16 cm) and test (33 cm) compartments, the latter of which contained two terra cotta flower pots arranged side-by-side against the rear wall. Behavior was scored by an experimenter blind to drug treatment using a video feed to a TV monitor that allowed the rats to be viewed through the rear wall of the test compartment.

Initially, rats were shaped to dig for a food reward (1/4 of a Froot Loop, Kellog’s, Battle Creek, MI, USA) buried at varying depths in the pots, which were filled with home cage bedding (wood shavings). Raising the Plexiglas barrier marked the start of each trial and rats were considered shaped to dig when they successfully obtained the food reward buried 2 cm below the surface of both pots in under 2 min.

For discrimination problems, pots were filled with clean home cage bedding and the rims of the pots were scented with two different odorants (e.g., rose+ and citrus−). Odorants used were perfume oils obtained from The Bath Junkie and The Body Shop and 10 μl of the full strength oil was applied to pots. Novel odors were used for each discrimination problem (i.e.—each odor was used only once). Only one pot contained the food reward (+), and the odor of the food was disguised by crushed Froot Loops sprinkled over the bedding filling both pots. The position (left or right) of the rewarded pot was varied pseudo-randomly across trials. Criterion performance on each problem consisted of six consecutive trials in which the correct (baited) pot was chosen. Rats were considered shaped to discriminate after reaching criterion performance on two odor problems prior to the onset of pharmacological testing.

Pharmacological testing began on a separate day after completion of shaping. Young and aged rats received i.p. injections of one of three doses of the GABA(B) receptor antagonist CGP55845 (0.001, 0.01 or 0.1 mg/kg; Tocris, Ballwin, MO, USA) or 0.9% saline vehicle alone (1 ml/kg) 40 min prior to testing. The number of trials to reach criterion was used as the measure of performance. The order of injections was as follows: CGP55845, saline, CGP55845, saline, CGP55845, saline, with the order of presentation of the doses of CGP55845 randomized across rats and age groups. Only one dose of CGP55845 or saline was given each day and a 48 h washout period was interposed between injections (during which no testing was conducted). A second cohort of animals was tested with an additional .001 mg/kg dosage. This dosage was added to provide a more comprehensive dose response curve after it was clear in the initial cohort that both 0.01 and 0.1 mg/kg doses significantly improved performance.

After completion of discrimination testing, a subset of rats were tested for their ability to detect and respond to decreasing concentrations of odorants following vehicle (saline) and the highest effective dose of CGP55845 (0.1 mg/kg). Significant attrition