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EFFECTS OF SUSTAINED PRONGF BLOCKADE ON ATTENTIONAL CAPACITIES IN AGED RATS WITH COMPROMISED CHOLINERGIC SYSTEM

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Abstract—Disruption in nerve growth factor (NGF) signaling via tropomyosin-related kinase A (trkA) receptors compromises the integrity of the basal forebrain (BF) cholinergic system, yielding cognitive, specifically attentional, impairments in Alzheimer's disease (AD). Although normal aging is considered a risk factor for AD, the mechanisms underlying the selective vulnerability of the aging cholinergic system to trkA disruption is not clear. The levels of proNGF, a proneurotrophin that possesses higher affinity for p75 receptors, increase in aging. The present study was designed to test the hypothesis that cholinergic and attentional dysfunction in aged rats with reduced BF trkA receptors occurs due to the overactivation of endogenous proNGF signaling. We employed a viral vector that produced trkA shRNA to suppress trkA receptors in the corticopetal cholinergic neurons of aged rats. BF trkA suppression impaired animals' performance on signal trials in both the sustained attention task (SAT) and the cognitively taxing distractor version of SAT (dSAT) and these deficits were normalized by chronic intracerebroventricular administration of proNGF antibody. Moreover, depolarization-evoked acetylcholine (ACh) release and the density of cortical cholinergic fibers were partially restored in these animals. However, SAT/dSAT scores reflecting overall performance did not improve following proNGF blockade in trkA knockdown rats due to impaired performance in non-signal trials. Sustained proNGF blockade alone did not alter baseline attentional performance but produced moderate impairments during challenging conditions. Collectively, our findings indicate that barring proNGF-p75 signaling may exert some beneficial effects on attentional capacities specifically when BF trkA signaling is abrogated. However, endogenous proNGF may also possess neurotrophic effects and blockade of this proneurotrophin may not completely ameliorate attentional impairments in AD and potentially hinder performance during periods of high cognitive load in normal aging.

Key words: aging, acetylcholine, proNGF, attention, Alzheimer's disease.

INTRODUCTION

Basal forebrain (BF) cholinergic neurons located in the nucleus basalis of Meynert (nBM) and the substantia innominata (SI) project to all cortical areas and layers throughout the brain. Substantial evidence suggests that the integrity of cortical cholinergic inputs is necessary for normal attentional performance, and that such performance robustly activates cortical acetylcholine (ACh) release (McGaughy et al., 1996; Everitt and Robbins, 1997; Passetti et al., 2000; Arnold et al., 2002; Dalley et al., 2004; Sarter and Parikh, 2005; Parikh et al., 2007). Attentional impairments constitute the core components of global cognitive decline observed in Alzheimer's disease (AD; Perry and Hodges, 1999). Moreover, the cortical cholinergic input system undergoes extensive degeneration in AD that correlates with the severity of cognitive symptoms and disease duration (Counts et al., 2004; Mesulam, 2004; Counts and Mufson, 2005). Aging is a well-recognized risk factor for AD. Although the BF cholinergic system is highly vulnerable in aging (Casu et al., 2002), the contribution of age in cholinergic and cognitive decline associated with AD is not well defined.

BF cholinergic neurons require nerve growth factor (NGF) for trophic support (Mobley et al., 1986; Oosawa et al., 1999; Sofroniew et al., 2001). NGF-mediated signaling via a high-affinity tropomyosin-related kinase A (trkA) receptor is crucial for the development, maturation and function of these neurons (Li et al., 1995; Fagan et al., 1997; Huang and Reichardt, 2003). NGF also acts on another non-specific neurotrophin receptor, p75, which belongs to the tumor necrosis receptor family, and induces apoptotic signaling via a coreceptor, sortilin (Chao, 2003; Schor, 2005; Volosin et al., 2006; Clewes et al., 2008). Postmortem studies supported the hypothesis that disruption of trkA receptor function and possibly an imbalance between trkA/p75 signaling may contribute to the degeneration of BF cholinergic neurons leading to cognitive decline in AD (Mufson et al., 2000,

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Abbreviations: AA, ascorbic acid; AAV, adeno-associated viral; ACh, acetylcholine; AD, Alzheimer's disease; ANOVA, analysis of variance; BF, basal forebrain; ChAT, choline acetyltransferase; CHT, choline transporter; DA, dopamine; DAB, 3-3′-diaminobenzidine; dSAT, distractor version of SAT; GFP, green fluorescent protein; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; ITI, intertrial interval; LOD, limit of detection; LSD, least significant difference; nBM, nucleus basalis of Meynert; NGF, nerve growth factor; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PFC, prefrontal cortex; SAT, sustained attention task; SEM, standard error of the mean; SI, substantia innominata; SIBR, synthetic inhibitory BIC-derived RNA; TBS, tris-buffered saline; TBST, TBS containing 1% triton X 100; trkA, tropomyosin-related kinase A.

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2008; Counts et al., 2004; Counts and Mufson, 2005). However, recent studies utilizing conditional mutants show that region-specific deletion of the trkA gene does not affect the survival of BF cholinergic neurons (Sanchez-Ortiz et al., 2012) and cognitive performance of young and middle-aged animals (Müller et al., 2012). Moreover, we previously demonstrated that chronic suppression of BF trkA receptors produces cholinergic and attentional deficits in aged but not young rats (Parikh et al., 2013). Collectively, these findings indicate that aging interacts with preexisting abnormalities in trophic signaling to trigger cholinergic and cognitive decline as observed in AD.

NGF is secreted in the central nervous system as proNGF precursor that is synthesized as 25- and 32-kDa isoforms (Fahnestock et al., 2004; Bruno and Cuello, 2006). ProNGF undergoes proteolytic cleavage either intracellularly by proprotein convertases or extracellularly by plasmin to produce mature NGF (Edwards et al., 1988; Seidah et al., 1996; Bruno and Cuello, 2006). The ratio of proNGF to NGF increases in normal aging and other pathological conditions, and this effect presumably occurs as a consequence of disruption in cleavage mechanisms (Hempstead, 2009). ProNGF possesses a higher affinity for p75 receptors and may trigger apoptosis by activating this receptor (Nykjaer et al., 2004). *In vitro* studies demonstrated that this proneurotrophin exerts neurotoxic effects on sympathetic and BF neurons isolated from aging rodents (Al-Shawi et al., 2007, 2008). Moreover, acute hippocampal injection of proNGF in aged rats produced atrophy of septal cholinergic neurons (Fortress et al., 2011). However the consequences of age-related accumulation of endogenous proNGF on cholinergic signaling and attentional capacities have remained unknown. Here we examined the effects of chronic intracerebroventricular (i.c.v.) infusions of proNGF antibody (proNGF Ab) on cholinergic function in normal and BF trkA-suppressed aged rats. We employed a viral vector-based RNA interference strategy to knockdown trkA receptors specifically in the nBM/SI region of the BF (Parikh et al., 2013). This strategy was adopted to model decreases in trkA but not p75 receptors that is observed in AD patients (Counts et al., 2004), as well as to circumvent behavioral confounds produced by trkA reduction elsewhere in the nervous system (Holtzman et al., 1995; Luther and Birren, 2009). Our results demonstrate that proNGF blockade provides a partial rescue of cholinergic and attentional deficits in aged rats with lower levels of BF trkA receptors. Moreover, endogenous proNGF is not detrimental to the aging cholinergic system provided trophic signaling remains intact.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats (retired breeders; 10–12 months) were acquired from Charles River Laboratories (Malvern, PA, USA). The animals were housed in a temperature- and humidity-controlled facility with a 12-h light/dark cycle (lights ''on'': 7:00 AM) and had free access to food and water. Rats were maintained until 20 months of age following which training in an operant attentional task was initiated (see behavioral procedures as described in ''Behavioral training and testing''). Water access to animals was restricted to a 10-min period in the home cage following each behavioral session. Operant training took place 6 days/week. On non-training days, water access was increased to 30 min. Rats were individually housed and food was available *ad libitum* throughout the behavioral training and testing. The experiments were conducted in accordance with the National Institute of Health guidelines and were approved by the Institutional Animal Care and Use Committee, as well as the Institutional Biosafety Committee, at Temple University.

Behavioral training and testing

Apparatus. Rats were trained in operant chambers encased in sound-attenuating boxes each containing a fan to provide ventilation and low-level background noise (Med Associates Inc., St. Albans, VT, USA). Each chamber was equipped with two retractable levers, a central panel consisting of three panel lights (2.8 W each), a liquid receptacle attached to a water dispenser, and a house light (2.8 W) located on the rear wall. All events including the signal delivery, lever presentations, and water dispense were transmitted using programs written in Medstate notation via SmrtCtrl interface running through MED-PC software on a Dell Optiplex 960 computer.

Operant sustained attention task (SAT). Partially water-deprived rats were trained in operant SAT as described previously (Demeter et al., 2008; St Peters et al., 2011; Parikh et al., 2013). Briefly, rats were initially autoshaped on a FR-1 schedule of reinforcement to attain the lever press response and subsequent reward (0.02-mL water). To deter a side bias, lever presses on the dominant lever (i.e. the lever with $\geqslant 5$ presses) ceased to be reinforced until the discrepancy was reduced. Once the rats made 120 lever presses within a session, they were moved to the next phase of training, which required discrimination between signal (illumination of the central panel light for 1 s) and non-signal (no illumination) events. Each event was followed by the presentation of two levers 2 s later; levers remained extended for 4 s or until a lever press occurred. If no response was made during the 4-s lever presentation, an omission was recorded and the intertrial interval (ITI; 12 ± 3 s) was reinstated. On signal trials, a left lever press was scored as a ''hit'' and rewarded; an incorrect response (depression of the right lever) was deemed a ''miss''. During nonsignal trials, a right lever press was scored as a ''correct rejection'' and reinforced, while a left lever press was considered a ''false alarm''. The animals were not rewarded for incorrect responses. The presentation of signal and nonsignal trials were pseudo-randomized. Half of the animals in a group were trained with the reverse set of rules.