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Greater glucocorticoid receptor activation in hippocampus of aged rats sensitizes microglia

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ABSTRACT

Healthy aging individuals are more likely to suffer profound memory impairments following an immune challenge than are younger adults. These challenges produce a brain inflammatory response that is exaggerated with age. Sensitized microglia found in the normal aging brain are responsible for this amplified response, which in turn interferes with processes involved in memory formation. Here, we examine factors that may lead aging to sensitize microglia. Aged rats exhibited higher corticosterone levels in the hippocampus, but not in plasma, throughout the daytime (diurnal inactive phase). These elevated hippocampal corticosterone levels were associated with increased hippocampal 11β-hydroxysteroid dehydrogenase type 1 protein expression, the enzyme that catalyzes glucocorticoid formation and greater hippocampal glucocorticoid receptor (GR) activation. Intracisternal administration of mifepristone, a GR antagonist, effectively reduced immune-activated proinflammatory responses, specifically from hippocampal microglia and prevented *Escherichia coli*–induced memory impairments in aged rats. Voluntary exercise as a therapeutic intervention significantly reduced total hippocampal GR expression. These data strongly suggest that increased GR activation in the aged hippocampus plays a critical role in sensitizing microglia.

1. Introduction

Cognitive declines that occur with aging have recently been linked to age-related potentiation of neuroinflammatory responses to challenge, largely mediated by sensitized microglia (Norden and Godbout, 2013). In particular, aging sensitizes hippocampal microglia such that immune challenges result in a potentiated and protracted inflammatory response in the hippocampus, relative to that in young adult subjects (Barrientos et al., 2009a; Cunningham et al., 2005; Frank et al., 2010a; Godbout et al., 2005). This potentiated inflammatory response in aged rats has been associated with impairments in hippocampal long-term potentiation (Chapman et al., 2010), reduced expression of brain-derived neurotrophic factor (Chapman et al., 2012; Cortese et al., 2011), and long-lasting impairments in contextual and spatial forms of memory that depend on an intact hippocampus (Barrientos et al., 2006). In the following series of experiments, we investigated the factors that are responsible for aging-induced sensitization of hippocampal microglia. Our findings have led us to the somewhat paradoxical conclusion that age-related increases in basal hippocampal corticosterone (CORT), the principal glucocorticoid in rodents, is a key mediator of a microglia-dependent proinflammatory state in the hippocampus.

CORT is involved in metabolic function, immune reactions, and stress responses and is well known for its anti-inflammatory and immunosuppressant effects in both humans and animals (Selye, 1955). However, a more complex view that CORT actually has pleiotropic actions has emerged over the last dozen years (Busillo et al., 2011; Sorrells and Sapolsky, 2007; Sorrells et al., 2009). A series of articles (Dinkel et al., 2003; MacPherson et al., 2005; Munhoz et al., 2006; Sapolsky, 1999; Sapolsky and Pulsinelli, 1985) demonstrated that chronic exposure to stress levels of CORT exacerbated, rather than inhibited, the neuroinflammatory

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response to challenge. Others have since reported similar findings (de Pablos et al., 2006; Johnson et al., 2002). More recently, Frank et al. (2010b) reported that CORT administered acutely before a bacterial immune challenge facilitated the inflammatory response to the challenge, both in the periphery and in the brain. In contrast, CORT administered after the same immune challenge resulted in suppression of the inflammatory response. Together, these findings suggest that the temporal relationship between CORT elevations and immune challenge may be an important factor in determining whether CORT facilitates or suppresses the inflammatory response. They further demonstrated that prior in vivo administration of CORT potentiated the proinflammatory response of isolated hippocampal microglia exposed, *ex vivo*, to an immune challenge (Frank et al., 2010b). These data established that elevations in CORT can be a key factor in sensitizing hippocampal microglia.

These findings led us to hypothesize that aging might increase basal concentrations of CORT, and that this factor may be causally related to aging-induced microglial sensitization and cognitive decline. To investigate this possibility, we used a multidisciplinary approach to fully characterize basal CORT and glucocorticoid receptor (GR) activation in young and old rats across times of day. Reducing GR activation with central mifepristone administration normalized hippocampal, microglial, immunophenotype, and immune-activated proinflammatory responses and prevented *Escherichia coli*—induced memory impairments in aged rats. Finally, voluntary exercise was shown to be an effective behavioral therapeutic to reduce hippocampal GR expression. Taken together, this series of studies strongly suggests that greater GR activation in the hippocampus of aged rats is a key factor in sensitizing hippocampal microglia, as reduction of this activation reduced neuroinflammatory responses and prevented long-lasting memory deficits following an immune challenge.

2. Methods

2.1. Subjects

Subjects were male F344xBN F1 rats obtained from the National Institute on Aging Rodent Colony maintained by Harlan (Indianapolis, IN). On arrival at our facility, aged rats were 24 months old and weighed approximately 550 g. Young adult rats were 3 months old and weighed approximately 275 g. All rats were age matched and housed 2 to a cage (52 L × 30 W × 21 H in cm). The animal colony was maintained at 22 °C ± 1 °C on a 12-hour light dark cycle (lights on at 07:00 hours). All rats were allowed free access to food and water and were given at least 1 week to acclimate to colony conditions before experimentation began. All experiments were conducted in accordance with protocols approved by the University of Colorado Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

2.2. Jugular vein cannulation

Rats were anaesthetized with inhaled isoflurane (5% initial and 2% maintenance). The right jugular vein was exposed and a silastic-tipped (Dow Corning, Midland, MI) polythene cannula (inner diameter, 0.5 mm; outer diameter, 0.93 mm; Portex, Hythe, UK) filled with heparinized (10 IU/mL), pyrogen-free isotonic saline was inserted into the vein until it was positioned close to the right atrium. The other end of the cannula was exteriorized through a scalp incision, and the neck incision was sutured. The free end of the cannula was inserted through a protective spring, which was fixed to the parietal bones using 2 stainless steel screws and dental cement. Following recovery from anesthesia, the rats were individually housed in a room housing the automated blood sampling system. The free end of the protective spring was attached to a liquid swivel (Instech Laboratories, Inc, Plymouth Meeting, PA) that rotated through 360° in the horizontal plane and up to 180° in the vertical plane, giving the rats maximum freedom of movement. The rats recovered 5 days before the experiment, and each day during the recovery period the jugular cannulae were flushed with heparinized saline to maintain patency.

2.3. Automated blood sampling

Automated blood sampling (ABS) procedures were used to collect blood samples for measurement of plasma corticosterone concentrations as previously described (Lowry et al., 2009). Five days following the surgery, the free ends of the jugular cannulae were attached to the ABS system using a liquid swivel. Flow of blood or heparinized saline between the rat, a reservoir, and a fraction collector was achieved by cooperative actions of a peristaltic pump and a 3-way valve controlled by a computer outside of the procedure room. Rats were connected to the system, and approximately 50 μL of diluted blood was taken every 30 minutes for 24 hours. Following every sample, the blood volume that was removed from the rat was replaced with heparinized saline to prevent reductions in blood volume. Experimenters did not enter the room throughout the collection period.

Because of limitations of the ABS system, there were variations in the amount of blood collected in individual samples. To account for this intra-sample variation the proportion of blood, relative to heparinized saline, that was collected for each sample was assessed. A volume of 3 μL was removed from each sample and, using a spectrophotometer (Synergy HT, BioTek Instruments, Winooski, VT), compared the samples with a standard curve of known blood proportions in sterile-heparinized saline (0%, 12.5%, 25%, 37.5%, 50%, 62.5%, 75%, 87.5 %, and 100%). Samples and standard curve were diluted 1:90 in sterile-heparinized saline and loaded into 96-well plates in triplicate and read at 630 nm wavelength. A linear regression line was fit to the standard curve to produce an equation that was used to solve for the unknown sample dilutions. The dilution of each sample was transformed into a coefficient that was applied to the hormone assay results to correct for different blood dilutions in samples. Blood samples containing less than 15% blood were not considered reliable for corticosterone enzyme immunoassay and were excluded from the analysis.

2.4. In vivo hippocampal microdialysis

A guide cannula for the microdialysis probe (CMA/Microdialysis, North Chelmsford, MA) was stereotaxically placed unilaterally within the dorsal hippocampus of young and aged rats, using the following coordinates relative to Bregma: Anterior/Posterior: −5.60 mm, Medial/Lateral: +4.9 mm, Dorsal/Ventral: −3.4 mm. The guide cannula was secured to the top of the skull with dental acrylic. A screw cap of a 15-mL conical centrifuge tube, whose central lid portion was removed, was also affixed to the skull with dental acrylic so that its threads were exposed and it encircled the cannula. This was done so that the skull assembly could be protected during microdialysis. Rats were given 1 week to recover before the experimentation began, and until that time, they were housed 2 to a cage. The night before sampling began, each animal was placed individually in a Plexiglas bowl (Bioanalytical Systems, West Lafayette, IN) to give it time to acclimate to the new conditions. Approximately 5 cm portion of a 15-mL Eppendorf tube was screwed onto the skull-mounted screw cap, through which the dialysis tubing, protected within a metal spring, entered, and attached to the probe. Rats were able to move freely within the Plexiglas bowl via a freely moving swivel arm attached to this assembly. The microdialysis probe (0.5 mm in diameter, 1 mm