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Brain monoamines and antidepressant-like responses in MRL/MpJ versus C57BL/6J mice

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ABSTRACT

The MRL/MpJ mouse demonstrates enhanced wound healing and tissue regeneration and increased neurotrophic mobilization to chronic antidepressant drug treatments. This study compared brain monoamine systems between MRL/MpJ and C57BL/6J mice as a potential basis for strain differences after chronic antidepressant treatment. MRL/MpJ mice had significantly higher tissue levels of serotonin and dopamine in multiple brain regions. Microdialysis studies demonstrated that baseline levels of extracellular serotonin did not differ between strains. However, acute administration of the selective serotonin reuptake inhibitor citalopram produced an increase in extracellular serotonin in the ventral hippocampus of MRL/MpJ mice that was twice as large as achieved in C57BL/6J mice. The greater effects in MRL/MpJ mice on 5-HT levels were not maintained after local perfusion of citalopram, suggesting that mechanisms outside of the hippocampus were responsible for the greater effect of citalopram after systemic injection. The density of serotonin and norepinephrine transporters in the hippocampus was significantly higher in MRL/MpJ mice. In addition, the expression of 5-HT_{1A} mRNA was lower in the hippocampus, 5-HT_{1B} mRNA was higher in the hippocampus and brainstem and SERT mRNA was higher in the brain stem of MRL/MpJ mice. The exaggerated neurotransmitter release in MRL/MpJ mice was accompanied by reduced baseline immobility in the tail suspension test and a greater reduction of immobility produced by citalopram or the tricyclic antidepressant desipramine. These data suggest that differences in the response to acute and chronic antidepressant treatments between the two strains could be attributed to differences in serotonin or catecholamine transmission.

1. Introduction

MRL/MpJ (MRL) mice are an inbred strain known to display extraordinary wound healing and dramatic tissue regeneration in response to different types of injury when compared to most other mouse strains, such as C57BL/6J (B6) mice (Clark et al., 1998; Leferovich et al., 2001). Recently, another set of phenotypic differences between MRL and B6 mice were identified, including enhanced responsiveness to the proneurogenic, neurotrophic and behavioral effects of chronic antidepressant treatments (Balu et al., 2009; Hodes et al., 2010). Similarly, MRL mice showed a greater increase of hippocampal cell proliferation than B6 mice after chronic wheel running that led to improved spatial memory (Thuret et al., 2009). There are a number of reasons for examining genetic variations and physiological mechanisms common between tissue repair mechanisms and the neurotrophic response to antidepressant treatments. Major depressive disorder has been associated with evidence for tissue injury in the brain, especially in the hippocampus (MacQueen and Frodl, 2011; Sheline et al., 1999), and with increased inflammation mediated by cytokines (Capuron and Miller, 2011). Finally, chronic antidepressant treatments augment mechanisms involved in neuronal plasticity in the hippocampus, such as neurotrophin signaling and neurogenesis, that can counteract these pathological features of depression (Duman and Monteggia, 2006; Wager-Smith and Markou, 2011).

A primary pharmacological effect of most antidepressants is their alteration of serotonin (5-HT) and noradrenergic transmission by inhibiting uptake mechanisms by their respective presynaptic transporters (Frazer, 1997; Sanchez and Hyttel, 1999). 5-HT and norepinephrine (NE) are known mediators of the inflammatory and

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cell proliferative stages of wound healing (Gosain et al., 2006; Malinin et al., 2004) and produce similar increases in neurotrophic mobilization and hippocampal neurogenesis following chronic administration of antidepressants (Balu and Lucki, 2009; Vaidya et al., 2007). Various behavioral tests in rodents are also used to evaluate compounds for potential antidepressant activity (Crowley and Lucki, 2005). One such paradigm, the tail suspension test (TST), has been shown to be sensitive to numerous antidepressants from different pharmacological classes, including those that augment 5-HT and NE transmission (Steru et al., 1985) and therefore has good predictive validity (Cryan et al., 2005). However, inbred and outbred mouse strains show large differences of performance on the TST (Crowley et al., 2005; Cryan et al., 2005).

The purpose of this study was to examine whether endogenous variations in brain monoamine systems between MRL and B6 mice could contribute to any phenotypic differences observed in their response to acute and chronic treatment with antidepressants. Therefore, tissue monoamine content and monoamine transporter densities and gene expression were compared between strains and *in vivo* microdialysis was used to confirm functional differences. Behaviorally, MRL and B6 mice were also compared in the TST to acute administration of two pharmacologically distinct antidepressant drugs. These studies established endogenous differences in monoamine transmission between MRL and B6 mice that could mediate their behavioral phenotypes and pharmacological sensitivity to antidepressant drugs.

2. Materials and methods

2.1. Animals

Adult male B6 and MRL (Jackson Laboratories, Bar Harbor, ME, USA) mice were 8–10 weeks old at the beginning of all studies. The animals were housed in groups of five in polycarbonate cages and maintained on a 12-h light/dark cycle (lights on at 07:00 h) in a temperature (22 °C)- and humidity-controlled colony. The animals were given free access to food and water. Animal procedures were conducted in accordance with the guidelines published in the NIH Guide for Care and Use of Laboratory Animals and all protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

2.2. Tissue monoamine analysis

Mice were decapitated and their brains quickly removed for dissection. The hippocampus, frontal cortex, amygdala, and brain stem were dissected, flash frozen and stored at −80 °C until preparation for analysis using high performance liquid chromatography (HPLC) with electrochemical detection.

Tissue samples were homogenized in 0.1 N perchloric acid with 100 μM EDTA (15 μl/mg of tissue) using a tissuemizer (Tekmar, Cleveland, OH, USA). Samples were centrifuged at 15,000 rpm (23,143 *g*) for 15 min at 2–8 °C. The supernatants were filtered using Costar Spin-X™centrifugal filters (Fisher Scientific, Pittsburgh, PA, USA) and then split into two aliquots. Samples (12 μl) were injected by an autosampler (Sample Sentinel, Bioanalytical Systems, West Lafayette, IN, USA) and analyzed in separate assays for tissue content of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and dopamine (DA) or for NE.

The HPLC separation for 5-HT consisted of a PM80 solvent delivery system and a 10 μl sample loop linked in series to a reversed phase microbore column (ODS 3 μm, 100°–1 mm; Bioanalytical Systems). The mobile phase for the separation of 5-HT consisted of 12.42 mM citric acid (Sigma), 39.85 mM $NaPO_4$ monobasic (Fluka, Buchs SG, Switzerland), 0.25 mM EDTA (Fluka), 0.737 mM 1-decanesulfonic acid (Sigma), 10 mM NaCl (Fluka), 0.2% triethylamine (Sigma), 16.5% methanol (Fisher Scientific) adjusted to a pH of 4.1. The flow rate through the system was 60 μl/min, and the detector was set at a potential of +0.60 V relative to a Ag/AgCl reference electrode.

The HPLC separation for NE and DA consisted of a PM80 solvent delivery system and a 10-μl sample loop linked in series to a reversed phase microbore column (ODS 5 μm, 150°—1 mm; Bioanalytical Systems). The mobile phase for the separation of NE and DA consisted of 14.5 mM NaH_2PO_4 (Fluka), 30 mM sodium citrate (Fluka), 27 μM disodium EDTA (EMD Chemicals, Gibbstown, NJ, USA), 10 mM diethylamine HCL (Sigma), 1.95 mM 1-decanesulfonic acid (Sigma), 8% acetonitrile (Fisher Scientific), 1% tetrahydrofuran (Fluka) adjusted to pH 3.4. Mobile phase was pumped through the system at 80 μl/min, and the detector was set at a potential of +0.65 V relative to a Ag/AgCl reference electrode.

Standard concentrations of 5-HT, NE and DA were prepared before injection of tissue samples. Tissue concentrations of monoamines were determined using a linear regression analysis of the peak heights obtained from a range of standards and expressed as pg/mg tissue.

2.3. Microdialysis procedures

Microdialysis probes were custom-made and surgically implanted as described previously (Knobelman et al., 2000). Mice were anesthetized with chloral hydrate (400 mg/kg, i.p.). The probe was implanted in the ventral hippocampus at the following coordinates: AP −2.8, ML ±3.2, and DV −5.0 mm from bregma (Franklin and Paxinos, 1997) using a stereotaxic instrument. Following surgery, the mice were placed into a 21.5 cm high, clear polycarbonate cylindrical *in vivo* microdialysis apparatus with a counterbalance arm holding a liquid swivel (Instech Laboratories, Plymouth Meeting, PA) and allowed to recover overnight.

Microdialysis experiments started 17–20 h after surgery. Dialysate samples were collected into polypropylene microcentrifuge vials at 20-min intervals. Four fractions were collected to measure baseline values before systemic administration of citalopram (20 mg/kg). Samples were collected for three additional hours after systemic drug challenge to compare the effects of fluoxetine on extracellular 5-HT levels between B6 and MRL mice. To examine the effects of citalopram given directly into the hippocampus, the perfusion media was changed from aCSF to citalopram (1 mM) after the collection of baseline samples using a liquid switch. Samples were stored at −80 °C until analyzed using a Bioanalytical Systems 460 High Pressure Liquid Chromatograph by a BAS Sample Sentinel Refrigerated Microsampler set to a 12 μl injection volume as described previously (Kreiss and Lucki, 1994). The 5-HT from chromatographs of dialysate samples were identified by comparing their elution times with those of reference standards. The amount of 5-HT in each dialysate sample was quantified from their respective peak heights using a linear regression analysis of the peak heights obtained from a series of reference standards.

At the completion of the experiment, brains were removed, placed in cold isopentane and frozen at −80 °C. The brains were then sectioned (35 μm) with a refrigerated cryostat, stained with Neutral Red, and the tissue examined for the location of the dialysis probe. No animals were excluded for improper probe placement.

2.4. Radioligand binding for SERT and NET

Binding methodology was adapted from previous studies (Dewar et al., 1991; Tejani-Butt, 1992). Tissues were homogenized in 50 mM Tris buffer (pH 7.4 at 24 °C) containing 400 mM NaCl and 5 mM KCl and centrifuged twice at 35,000 × *g* for 10 min in fresh buffer. The homogenates were added to tubes containing 10 nM [^{3}H] nisoxetine or 300 pM [^{3}H]paroxetine for measurements of the NE transporter or the 5-HT transporters, respectively. Incubations were carried out for 4 h at 4 °C and bound transporters were separated from free ligand by filtration over Whatman GF/C filters wet with 0.5% polyethylenimine and mounted on a Brandell Cell Harvester (Gaithersberg, MD). The filters were then counted in a liquid scintillation counter. Nonspecific binding to the NE or 5-HT transporter was determined in the presence of 10 μM desipramine or 30 μM citalopram, respectively, and specific binding was defined as the difference between total binding and nonspecific binding.

2.5. Quantitative real-time PCR

Mice were sacrificed and the hippocampal tissue was dissected from the surrounding cortex by sight. For sections of the brainstem, including the raphe nuclei, the whole brain was extracted and placed immediately in a mouse brain block (Kopf Brain Blocker, Ireland) on ice. Tissue slices through the brainstem were obtained and a round 1 mm punch taken from the region containing the raphé and stored immediately at −70° until use. RNA was isolated using the RNAqueous-4PCR kit for isolation of DNA-free RNA (Ambion, Applied Biosystems, Austin, TX) following the manufacturer's instructions. 500 ng of RNA was used to synthesize cDNA using the Superscript Vilo cDNA synthesis kit (Invitrogen, Carlsbad, CA). All reactions were performed using a master mix of SYBR green (Applied Biosystems, Austin, TX) and 300 nM OligoDt primers (final concentration, Operon, Huntsville, AL). Reactions (25 μl) were run using the Stratagene MX3000 and MXPro QPCR software. Cycling parameters were 95 °C for 10 min, followed by 40 cycles of 95 °C (30 s) and 60 °C (1 min), followed by a melting curve analysis. Reactions were performed in triplicate and the median cycle threshold was used for analysis and normalized to TATA binding protein (TBP). Primers for SERT: Forward 5′-GCTGAGCTGACTTGGATA-3′, Reverse 5′-ACAGACGTTCACAGACCTAA-3′. Primers for 5-HT_{1A}: Forward 5′-CTGTTTATCGCCCTGGATG-3′, Reverse 5′-ATGAGCCAAGTGAGCGAGAT-3′. Primers for 5-HT_{1B}: Forward 5′-TTCTTCATCATCTCCCTGGTG-3′, Reverse 5′-AGCGTATCAAGTTTGTGGACG-3′.

2.6. Tail suspension test

The tail suspension test (TST) was conducted using procedures described previously that measured the response to antidepressant drugs (Crowley et al., 2005). Mice were suspended by their tail with tape to a vertical aluminum bar connected to a strain gauge for 6 min. Mice were positioned so that the base of their tail was aligned with the bottom of the bar in order to decrease the propensity for mice to climb their tail during the test, particularly with B6 mice (Mayorga and Lucki, 2001). The TST measured the duration of behavioral immobility using an