

The β -adrenergic receptor blocker and anti-inflammatory drug propranolol mitigates brain cytokine expression in a long-term model of Gulf War Illness

Detailed Data Collection Methods

Gulf War Illness Exposure Paradigm and Treatment

Adult male C57BL/6J mice (n=5-7 mice per group), 8-12 weeks of age were purchased from Jackson Labs (Bar Harbor, ME). Mice were exposed to CORT (200 mg/L in 0.6% EtOH; Steraloids, Newport, RI, USA) in the drinking water for 7 days. On day 7, mice received a single intraperitoneal injection of DFP (4 mg/kg, MilliporeSigma, St. Louis, MO, USA) or physiological saline. After the initial exposure, mice received intermittent 7-day bouts of CORT water on days 14-21 and 28-35. On day 35, mice received a single subcutaneous injection of LPS (0.5 mg/kg, MilliporeSigma) or physiological saline. For animals receiving treatment, mice were given a single intraperitoneal injection of propranolol (20 mg/kg in saline) either outside of CORT (Day 24, Tx-) or during CORT (Day 31, Tx+). All animals were sacrificed by decapitation at 6 hours following LPS or saline exposure on Day 35.

Tissue Preparation

Immediately after decapitation, whole brains were removed from the skull with the aid of blunt curved forceps. Cortices and hippocampi were dissected free hand on a thermoelectric cold plate (Model TCP-2, Aldrich Chemical Co., Milwaukee, WI, USA) using a pair of fine curved forceps (Roboz, Washington, DC, USA). Brain regions were frozen at -85 °C and used for subsequent isolation of total RNA.

qRT-PCR

The total RNA from the hippocampus and cortex were isolated using Trizol® reagent (Thermo Fisher Scientific, Waltham, MA, USA) and Phase-lock heavy gel (Eppendorf, AG Hamburg, Germany), and purified using RNeasy mini-spin columns (Qiagen, Valencia, CA, USA). Total RNA (1 ug) was reverse transcribed to cDNA using Superscript III and oligo (dT)12-18 primers (Thermo Fisher Scientific, Waltham, MA, USA) in a 20 μ L reaction. Real-time PCR analysis of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase

(GAPDH), and of the proinflammatory mediators, TNF α , IL-6, CCL2, IL-1 β , leukemia inhibitor factor (LIF), and oncostatin M (OSM), as well as the astrocyte marker glial fibrillary acidic protein (GFAP) was performed in an ABI7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) in combination with TaqMan® chemistry as previously described. All PCR amplifications (40 cycles) were performed in a total volume of 50 μ L, containing 1 μ L cDNA, 2.5 μ L of the specific Assay of Demand primer/probe mix (Thermo Fisher Scientific, Waltham, MA, USA), and 25 μ L of Taqman® Universal master mix (Thermo Fisher Scientific, Waltham, MA, USA). Sequence detection software (version 1.7; Applied Biosystems/Thermo Fisher Scientific, Waltham, MA, USA) was used to determine threshold cycle (CT) values.