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Characterization of the temporo-spatial effects of chronic bilateral intrahippocampal cannulae on interleukin-1β

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Abstract

The implantation of a foreign object in the brain produces an acute neuroinflammatory state in which glia (astrocytes and microglia) may remain chronically activated in response to the inert foreign object. Activated glia can exhibit a sensitized pro-inflammatory response to immunogenic stimuli. This may be relevant to intracranial cannula implantation, which is commonly used to administer substances directly into the brain. If intracranial cannulation activates glia, a subsequent neuroinflammatory stimulus might induce a potentiated pro-inflammatory response, thereby introducing a potential experimental confound. We tested the temporal and spatial responses of interleukin-1β (IL-1β) to an acute immune challenge produced by lipopolysaccharide (LPS) in animals with chronic bilateral intrahippocampal cannulae implants (stainless steel). Cannulation increased the gene expression of the microglia activation antigens MHC II and CD11b, but not the astrocyte antigen GFAP. Moreover, this activation was temporally and spatially dependent. In addition, IL-1β mRNA, but not IL-1β protein, was significantly elevated in cannulated animals. Administration of LPS, however, significantly potentiated the brain IL-1β response in cannulated animals, but not in stab wounded or naïve animals. This IL-1β response was also temporo-spatially dependent. Thus, the pro-inflammatory sequelae of intracranial cannulation should be considered when designing studies of neuroinflammatory processes.

Keywords: Cannula; Interleukin-1; Glia; Lipopolysaccharide; GFAP; MHCII; CD11b; Method

1. Introduction

For the past 40 years, the use of cannula implants has been very well accepted. Injecting agents directly into the brain via a cannula is a useful approach to manipulate the function of a specific brain region without altering whole brain function. One of the earliest studies that validated this method, reported that animals can be ready for experimental use as early as 7 days after cannulation (Hilliard et al., 1968). This assumption was based on the motor activity and other behaviors of the cannulated animals compared to that of naïve animals. It has been implicitly assumed that the presence of a cannula does not induce an effect by itself. However, Hilliard et al. (1968) noted inflammation of the lateral ventricle, the site of cannula placement. At the time, it was not clear what induced the inflammation. However, it is now known that the implantation of a foreign object in the brain induces an inflammatory immune response, mediated primarily by glia (astrocytes and microglia) (Schultz and Willey, 1976; Stensaas and Stensaas, 1976; Szarowski et al., 2003; Turner et al., 1999). An acute immune response (<1 week) occurs, wherein the glial reaction is towards the mechanical trauma produced by the foreign object. The spread of glial activation to distant tissues is directly proportional to the size of the foreign object (Turner et al., 1999). Subsequently, a chronic immune response ensues characterized by marked glial mobilization towards the site of injury, where glia form an interwoven network to isolate the foreign object from healthy tissue (i.e. a glial scar). At 2 weeks, there is a marked alignment of glia along the foreign object and glial immunoreactivity is still detected at tissues distant from the injury site. By 4 weeks, this distant glial activation dissipates. By 6 weeks, only those glia that are strongly adhered to the foreign object maintain a level of activation and do so as long as the foreign object remains in situ (Turner et al., 1999). This temporal and spatial glial response is of critical importance

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because the durations mentioned (1, 2 and 4 weeks) are durations that researchers usually choose to allow animals to recover after implanting cannula(e).

Since cannulation is a frequently used method, it is important to determine whether intracranial cannulation can sensitize a central nervous system immune response to an immunogenic stimulus. The effects of cannulae implanted in the dorsal hippocampus were examined on the pro-inflammatory response, specifically interleukin-1β (IL-1β) to a peripheral immune challenge produced by LPS.

2. Materials and methods

2.1. Animals

Adult viral-free Sprague–Dawley male rats (350–400 g) (Harlan Labs, Madison, WI) were used for all experiments. They were housed in pairs on a 12/12 light/dark cycle (lights on at 07:00 h; room temperature 23 ± 3 °C) with food and water ad libitum. All animals were allowed to acclimate for 1 week. Experimentation began at 07:00 h and lasted between 4 and 5 h. All procedures were in accordance with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

2.2. Intrahippocampal cannulae implantation

Chronic indwelling stainless steel guide cannulae were implanted or used to induce a stab wound in the dorsal hippocampus using Watson and Paxinos rat brain atlas coordinates (AP: −3.5 mm; ML: +2.4 mm; DV: −3.0 mm, relative to Bregma) (Paxinos and Watson, 1986) (see Fig. 1A and B). Cannulae were secured using dental acrylic and a dummy injector (4 mm in length) was placed to maintain patency. Rats were allowed to recover for 1, 2, or 4 weeks.

2.3. Lipopolysaccaride (LPS) preparation and administration

LPS (*Escherichia coli*, serotype 0111: B4 Sigma Lot No. 072k4096) or equal volume 0.9% endotoxin-free saline (Abbott Laboratories, North Chicago, IL) was used. The dose used was 25 μg/kg. Lyophilized LPS (25 mg) was reconstituted with 0.9% sterile saline (10 ml) to derive a concentration of 2.5 mg/ml and then LPS was aliquoted in 100 μl vials and stored at −20 °C until used. At the time of experimentation, one 100 μl aliquot was thawed and added to 9.9 ml of 0.9% sterile saline yielding a concentration of 25 μg/ml. LPS for all experiments was administered intraperitoneally (i.p.).

2.4. Tissue collection

Animals were anesthetized using sodium pentobarbital (kg/ml). Prior to perfusion, cardiac blood was collected. Animals were transcardially perfused with 250 ml 0.9% chilled saline. Immediately after perfusions the brains were extracted and several brain regions were dissected. The tissues dissected were hippocampus (site of cannula placement), posterior cortex (peri-

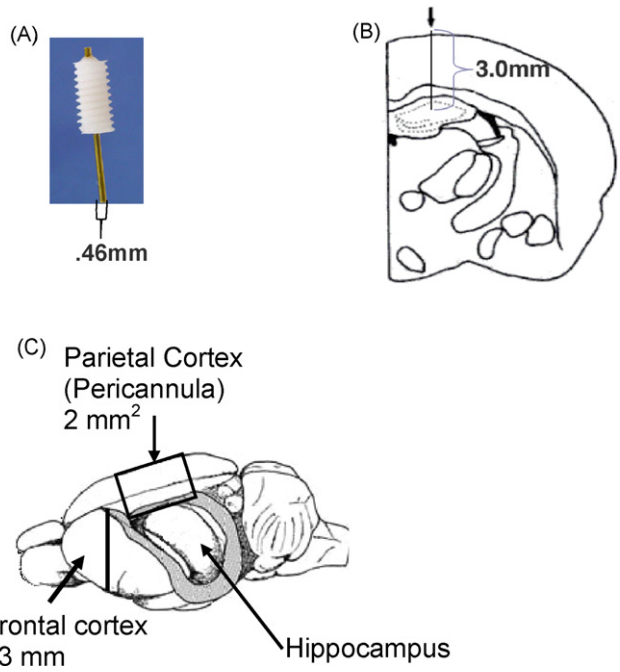

Fig. 1. Illustrations include the cannula used for these studies and the brain regions assessed for analyses of IL-1β. (A) Plastic One's stainless-steel cannula (3 mm × 0.46 mm diameter) were used to test the effects of cannulation on altering IL-1β levels. (B) Site of cannulation placement, dorsal hippocampus (CA1 region). (C) Brain regions dissected, parietal cortex (per-cannula tissue, ∼2 mm^2), hippocampus (left and right hemispheres), and frontal cortex (∼3 mm each hemisphere).

cannula tissue) and frontal cortex (tissue at a distant from injury site) (see Fig. 1C). The tissues were immediately flash frozen in liquid nitrogen and stored at −80 °C until assayed. To obtain both protein and mRNA measures from the same animal, left hemisphere of the hippocampus (and frontal cortex) was used for protein analyses and the right hemisphere for mRNA analyses.

2.5. Tissue preparation

To prevent tissue degradation, a buffer cocktail was prepared composed of three parts: (1) extraction buffer (190 ml Iscove's media, 10 ml fetal calf serum), (2) 20 mM phenylmethylsulfonyl fluoride (PMSF, Boehringer Manheim, 0.174 g PMSF and 50 ml isopropanol), and (3) enzyme cocktail (13.1 g amino-*n*-caproic acid (Sigma), 3.722 g ethylene-diamine-tetra-acetic acid (EDTA) (Mallinckrodt), and 0.783 g benzamidine (Sigma), in a total volume of 100 ml distilled water). The buffer cocktail was immediately added to the tissue-containing vial after retrieving from −80 °C. The tissues samples were sonicated (ultrasonic cell disruptor: Heat system, Inc., model #MS-50) for 10 s at a setting of 10 to liberate cell contents. The sonicated samples were centrifuged at 4 °C, 14,000 rpm for 10 min. The supernatants were extracted and stored at 4 °C until assayed.

2.6. Tissue assay

A rat IL-1β enzyme linked immunosorbent assay (ELISA) kits (R&D systems, cat #RLB00) was used to quantify the concentration of IL-1β in the supernatant. All procedures were