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Pro-inflammatory immune-to-brain signaling is involved in neuroendocrine responses to acute emotional stress

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ABSTRACT

Activation of the hypothalamo-pituitary-adrenal (HPA) axis by inflammatory stressors (e.g., bacterial lipopolysaccharide) is thought to involve vascular transduction of circulating cytokines, with perivascular macrophages (PVMs) along with endothelia, effecting activation of HPA control circuitry via inducible (cyclooxygenase-2- or COX-2-dependent) prostaglandin synthesis. To test the stressor-specificity of this mechanism, we examined whether ablation of PVMs or pharmacologic blockade of COX activity affected HPA responses to a representative emotional stressor, restraint. Exposing rats to a single 30 min acute restraint episode provoked increased plasma levels of at least one proinflammatory cytokine, IL-6, microglial activation and multiple indices of cerebrovascular activation, including COX-2 expression and increased brain prostaglandin E_2 levels at 0–2 h after stress. Pretreatment with the nonselective COX inhibitor, indomethacin, either icv (10 μg in 5 μl) or iv (1 mg/kg) significantly reduced restraint-induced Fos expression in the paraventricular hypothalamic nucleus (PVH) by 45%, relative to vehicle-injected controls. A 75% reduction of the PVH activational response was seen in rats exposed to acute restraint 5–7 days after ablation of brain PVMs by icv injection of liposomes encapsulating the bisphosphonate drug, clodronate. Basal plasma levels of ACTH and corticosterone were not altered in clodronate liposome-injected rats, but the peak magnitude of restraint-induced HPA secretory responses was substantially reduced, relative to animals pretreated with saline-filled liposomes. These findings support an unexpectedly prominent role for inducible prostaglandin synthesis by PVMs in HPA responses to acute restraint, a prototypic emotional stressor.

1. Introduction

It is now common among workers in the field to divide animal models of stress into two broad categories. Though various terminologies have been applied, what we refer to here as *physiological* stress involves targeted perturbations to the internal environment, while *emotional* stresses involve one or more exteroceptive sensory modalities, and include more prominent affective and cognitive components (Dayas et al., 2001a; Herman and Cullinan, 1997; Sawchenko et al., 2000). We have used systemic injection of the pro-inflammatory cytokine, interleukin-1 (IL-1), as a physiological stress model for exploring circuits and mechanisms by which immune activation associated with infection/inflammation activate a principal avenue of stress adaptation, the hypothalamo-pituitary-adrenal (HPA) axis (Ericsson et al., 1997, 1994; Schiltz and Sawchenko, 2002, 2007). Current evidence supports cerebrovascular transduction of circulating IL-1, with a macrophage-derived vascular cell type, termed here perivascular macrophages (PVMs), effecting activation of HPA control circuitry by their capacity to display inducible cyclooxygenase- (COX-2-dependent) prostaglandin synthesis (Choi and Bosetti, 2009; Schiltz and Sawchenko, 2003; Serrats et al., 2010).

Physical restraint, which typically involves close confinement of rats or mice in plastic cylinders for relatively brief intervals, is the most widely used emotional stress paradigm. Emotional stressors, including restraint, share with the cytokine model a capacity to engage the HPA axis and a major source of neuronal input to it provided by catecholaminergic neurons of the caudal medulla (Helfferich and Palkovits, 2003; Li and Sawchenko, 1998; Pezzone et al., 1993), but whereas IL-1-induced HPA responses require the integrity of this afferent source (Ericsson et al., 1994; Schiltz

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and Sawchenko, 2007), emotional stress-induced axis activation does not (Dayas et al., 2001b; Li et al., 1996; Ritter et al., 2003; Schiltz and Sawchenko, 2007). Despite this clear dissociation in the mediation of HPA responses to categorically distinct stressors, there is evidence to support a potential involvement of inflammatory mechanisms in acute emotional stress. This includes the observations that restraint results in increased circulating levels of at least one proinflammatory cytokine, interleukin-6 (IL-6; (Nukina et al., 1998; Takaki et al., 1994; Zhou et al., 1993) and gives rise to widespread activation of microglia (Nair and Bonneau, 2006; Sugama et al., 2007, 2009), the main intrinsic immune effector cells of the CNS. In addition, in a transcriptional profiling study comparing hypothalamic genes responsive to an IL-1 related immune stimulus (bacterial lipopolysaccharide; LPS) versus restraint, the emotional stressor upregulated as many inflammatory-related genes as did the immune one, with the two sets of responsive genes being almost completely non-overlapping (Reyes et al., 2003).

In the course of recent work assessing the effects of ablating PVMs, the vascular cell type implicated as a source of prostanoids for driving HPA and other centrally mediated acute phase responses to IL-1 or LPS (Serrats et al., 2010), we noted that this manipulation also attenuated CNS responses to acute restraint stress, which was originally intended to represent a control condition. Based on this unexpected result, we undertook to test the hypothesis that the mediators and mechanisms implicated in the HPA axis response to a systemic inflammatory challenge may to some degree generalize to the categorically distinct, emotional, insult posed by acute restraint stress. This involved identifying whether and which major proinflammatory cytokine may be elevated in the circulation of acutely restrained rats, and which vascular and/or inflammatory cell type(s) and markers may be impacted. Evidence implicating COX-dependent production of prostaglandin E_2 (PGE_2) by PVMs in the HPA responses was then tested experimentally using pharmacologic and liposome-mediated ablation techniques

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley albino rats (260–340 g) were used in most experiments. They were housed individually in a temperature-controlled room on a 12:12 h light/dark cycle with food and water freely available, and were adapted to handling for at least 5 d prior to any manipulation. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Salk Institute.

2.2. Restraint stress

Rats adapted to handling were placed in ventilated Plexiglas restrainers (Harvard Apparatus, Holliston, MA) in the morning (9:00 A.M) for 30 min in their home cages. Upon removal from restrainers, they remained in their home cages until the time of sacrifice, 0–3 h later. Control animals were handled comparably, but were not restrained.

2.3. Intravenous (iv) administration of indomethacin

Indwelling jugular catheters (PE50) containing sterile heparin-saline (50 U/ml) were implanted (Ericsson et al., 1994) under ketamine-xylazine-acepromazine anesthesia (25:5:1 mg/kg, sc). The sealed catheter was positioned with its internal Silastic® (Dow Corning, Midland, MI) tip at the atrium, and was exteriorized at an interscapular position. After 2 days' recovery, awake and freely moving rats were ready for the experimental protocol. Groups of rats (n = 6) were administered a 30 μl injection of the non-selective cyclooxygenase inhibitor, indomethacin (10 mg/kg), or vehicle (0.04 M PBS with 10% ethanol and 0.1% ascorbic acid, pH 7.4) through an indwelling jugular catheter (see above). Fifteen min after iv injection of indomethacin or vehicle, they were placed in the restrainers for 30 min as above, and remained in their home cages until they were anesthetized and perfused for histology.

2.4. Intracerebroventricular (icv) indomethacin injections

Groups of rats (n = 6) were anesthetized with ketamine/xylazine/acepromazine and stereotaxically implanted with 26 ga guide cannulae (Plastics One) terminating within a lateral ventricle. The cannulae were affixed to the skull with dental acrylic adhering to jewelers screws partially driven into the skull and were sealed with dummy stylets cut to terminate flush with the tip of the cannula. Five to 7 d later, the stylets were removed and replaced with 30 ga injector that extended 1.0 mm beyond the tip of the guide. Rats were given icv injections of indomethacin (10 μg in 5 μl), or vehicle (10% ethanol in 0.04 M PBS, pH 7.4) through the injection cannula connected to a 50 μl Hamilton syringe with PE 50 tubing. Fifteen min after central injection, animals were subjected to restraint stress as above.

2.5. Liposome preparation and delivery

Liposomes were prepared following the protocol described by N. van Rooijen (van Rooijen and Sanders, 1994). Briefly, these are polylamellar phosphatidylcholine/cholesterol membranes that encapsulate clodronate (used at a concentration of 250 mg/ml) or PBS, are mannosylated, to facilitate receptor-mediated uptake, and can be labeled with carbocyanine dye, DiI (D282, Molecular Probes), to enable detection of cells that have incorporated them. For icv injection, rats were anesthetized as above and mounted in a stereotaxic frame. Control (encapsulating PBS) or clodronate-liposomes were gently shaken to resuspend them, equilibrated to room temperature and injected in a volume of 50 μl over 10 min into a lateral ventricle using a 26 ga needle mounted onto stereotaxic arm and attached via PE tubing to a 1000 μl gastight syringe (Bee Stinger, BAS, n = 7, respectively). Rats used for experimentation 5–7d after surgery, at which time depletion of brain macrophages is maximal, and prior to any substantial repopulation from bone marrow-derived progenitors (Polfliet et al., 2001; Serrats et al., 2010).

2.6. Perfusion and histology

At appropriate time points, rats were anesthetized and perfused via the ascending aorta with 4% paraformaldehyde in 0.1 M borate buffer, pH 9.5, and the brains were removed, postfixed for 3 h, and cryoprotected overnight. Regularly spaced series of 30 μm-thick coronal sections were collected in cryoprotectant solution and stored at −20 °C until processing.

2.7. Immunohistochemistry

Fos protein was detected using a rabbit polyclonal antiserum raised against the synthetic peptide, SGFNADYEASSSRC, corresponding to 4–17 residues of human Fos protein (Ab-5, Lot 4191-1-1; Oncogene Sciences, Uniondale, NY), used at a 1:10,000 dilution. COX-2-immunoreactivity was detected with an antisera raised in goat against a peptide corresponding to amino acids 584–604 at the C terminus of the rat COX-2 precursor (Santa Cruz Biotechnology) used at a dilution of 1:5000–1:10000. To detect microglial cells, sections were incubated with antisera for the