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Relationships among the behavioral, noradrenergic, and pituitary–adrenal responses to interleukin-1 and the effects of indomethacin

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Abstract

Peripheral administration of interleukin-1 (IL-1) is known to activate the hypothalamo–pituitary–adrenal axis (HPA axis) and brain noradrenergic systems. We studied the relationship between these responses using in vivo microdialysis to assess the release of hypothalamic norepinephrine (NE), while simultaneously sampling blood for ACTH and corticosterone, and monitoring body temperature and behavior in freely moving rats. Rats were implanted with microdialysis probes in the medial hypothalamus, with intravenous catheters, and with telethermometers in the abdomen. Each rat was injected with saline and IL-1β (1 μg ip) in random order, monitoring microdialysate NE, body temperature and plasma ACTH and corticosterone for 2–4 h after injection. Saline injections were followed by transient increases in microdialysate NE and in plasma ACTH and corticosterone. IL-1β injections resulted in prolonged elevations of microdialysate NE, as well as plasma ACTH and corticosterone, and body temperature. IL-1β also induced shivering and a prolonged depression of locomotor activity. Pretreatment with indomethacin (10 mg/kg sc) prevented the IL-1β-induced increases in body temperature and the apparent increase in hypothalamic NE release, but only attenuated the IL-1β-induced shivering and the increase in plasma ACTH. The results indicate a close temporal relationship between the release of NE and HPA axis activation. Such a relationship is also supported by the similar effects of indomethacin pretreatment on NE and ACTH. The shivering is likely involved in the increase in body temperature, but indomethacin only attenuated the shivering while it blocked the fever. However, the effects of indomethacin clearly indicate that neither the increase in body temperature nor the increase in hypothalamic NE release was essential for HPA axis activation. These results suggest that hypothalamic NE is involved in the IL-1-induced HPA axis activation, but that this is not the only mechanism by which the HPA axis is activated by intraperitoneally injected IL-1.

Keywords: Interleukin-1; HPA axis; Norepinephrine; Behavior; ACTH; Temperature; Corticosterone; Microdialysis

1. Introduction

Peripheral administration of interleukin-1 (IL-1) to rodents has been shown to induce fever (Dinarello, 1984), to activate the hypothalamo–pituitary–adrenal (HPA) axis (Besedovsky et al., 1986), to induce certain behavioral changes (Dantzer et al., 2001; Larson and Dunn, 2001; McCarthy et al., 1985), and to affect noradrenergic and serotonergic neurotransmission in the brain (Dunn, 1988; Kabiersch et al., 1988). The mechanisms involved in these various responses have been studied extensively, nevertheless, the relationships among them remain unclear. For example, it is possible that the changes in body temperature underlie the changes in noradrenergic neuronal activity, the activation of the HPA axis, and/or the behavior. Alternatively, it is possible that the changes in the activity of noradrenergic neurons underlie the fever, the HPA axis activation, and/or the behavioral changes.

We sought to determine the relationships among and between these various responses to IL-1 by studying them simultaneously. We reasoned that a critical factor was the temporal relationships among the various responses, and

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that by using interventions known to impair the responses we could obtain important data on their interrelationships. Therefore, we studied six of these responses simultaneously in awake rats following intraperitoneal (ip) administration of rat IL-1β. We monitored changes in body temperature using telethermometers implanted in the abdomen, used in vivo microdialysis to assess the release of norepinephrine (NE) from the medial hypothalamus, while sampling blood for ACTH and corticosterone, and scoring behavior in awake unrestrained rats.

Because cyclo-oxygenase (COX) inhibitors have been shown to prevent the IL-1-induced fever (Blatteis and Sehic, 1997, 1998), and to impair the HPA axis responses (Dunn and Chuluyan, 1992; Katsuura et al., 1988; Krymskaya et al., 1987; Rivier and Vale, 1991), we assessed the involvement of COX in these various responses by pretreating the rats with the non-selective COX inhibitor, indomethacin.

2. Materials and methods

2.1. Experimental animals

The experiments were performed using male Sprague–Dawley rats, weighing 250–300 g obtained from Harlan Sprague–Dawley, Houston, TX. The animals were housed individually, under controlled environmental conditions of temperature (22 ± 2 °C), humidity (55 ± 5%) and on a 12:12 light cycle (lights on at 07:00 AM). Water and Purina rat chow were available ad libitum.

2.2. Materials

Rat interleukin-1β was purchased from R&D Systems (Minneapolis, MN). All other chemicals were of analytical grade from Sigma Chemical (St. Louis, MO).

2.3. Microdialysis probes

Concentric microdialysis probes were used. The inner fused silica tube (outer diameter 150 μm: Polymicrotechnologies, Phoenix, AZ) was inserted through a polyethylene tube (Clay Adams PE-50) and then inserted into a stainless steel injector (G-312 I: Plastics One, Roanoke, VA), the upper end of which was slipped into the PE-50 tubing in such way, that the fused silica passed through it. The dialysis membrane 3 mm long and 250 μm diameter (Cuprophane Pore Fiber, molecular weight cutoff 5–6000) was attached to the end of the stainless steel tube and sealed at its tip with epoxy cement (Locktite Quickset gel). The total length of the microdialysis probes was 19.4–19.8 mm, so that the probe tip was approximately 9.6 mm from the surface of the skull. The net active length of the dialysis membrane was approximately 2 mm.

2.4. Surgical procedure

Animals were anesthetized using Innovar Plus, (3 mg fentanyl, 210 mg droperidol, and 150 mg midazolam dissolved in 174 ml of water) at a dose of 6 μl/g body weight ip. The rat was placed on its back, a short incision (1.5 cm long) was made in the abdomen, and a telethermometer was placed into the abdominal cavity. The abdomen muscles and skin were then sutured. Next, a jugular vein catheter was implanted. A short (1.5 cm) incision was made in the neck, the jugular vein was carefully exposed and ligated with surgical silk as distally as possible to prevent bleeding. A small incision was made in the vein and a catheter (4 cm long) filled with heparinized saline (80 IU in 1 ml) was inserted into the vein. A small amount of blood was then withdrawn to check the position of catheter and the catheter was held in place with surgical silk. The other end of the catheter (10 cm long, equipped with a pedestal; Plastic One, Roanoke, VA) was passed beneath the skin and fixed to the skull with dental cement. The skin was sutured and topical antibiotic (Neosporin) applied.

The rats were then placed in a Kopf stereotaxic apparatus, and guide cannulae for microdialysis probes (C312G, 5.8 mm long: Plastics One) were implanted bilaterally and fixed to the skull using Cranioplastic cement (Plastics One). The coordinates for the microdialysis guide cannulae in the medial hypothalamus were: A–P: −2.0 mm; L: ± 2.2 mm; V: −4.8 mm, tilted medially at an angle 15.8° to prevent damage to the superior sagittal sinus. To enable the mounting of the microdialysis probes, stainless steel miniature self-tapping bone screws (Fine Scientific Tools, Foster City, CA) were also inserted in the skull and fixed with Cranioplastic cement. These procedures were approved by the Louisiana State University Health Sciences Center Animal Care and Use Committee and conform to National Institutes of Health guidelines.

2.5. Microdialysis system

The inflow to the microdialysis probe was driven by a CMA/100 Microinjection Pump. The perfusion fluid was artificial cerebrospinal fluid (aCSF) made according to Sharp et al. (1989): 1.2 mM $CaCl_2$, 1.2 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, 3.4 mM KCl, 140 mM NaCl, pH 7.2. Dialysate samples were collected in 20-min periods at a flow rate of 1.2 μl/min directly into 0.5 ml polypropylene vials containing 50 pg of *N*-methyldopamine (NMDA) as an internal standard in 5 μl of 0.15 M $HClO_4$–0.15 mM ethylenediaminetetraacetic acid (EDTA). The samples were frozen immediately after collection and stored on dry ice in a −70 °C freezer until analyzed.

2.6. HPLC

HPLC with coulometric detection was used to determine the content of NE and NMDA with reference to freshly diluted standards (Sigma Chemical). The system consisted of a chromatographic syringe pump (ISCO Model 100DM), an ESA Coulochem III detector (ESA, Chelmsford, MA), and a manual injector (Rheodyne 9126). Separation was performed on a Keystone microbore analytical 125 × 1 mm column C-18, particle size 5 μm (Thermo Hypersil). The mobile phase contained 50 mM sodium acetate, 0.5 mM EDTA, and 2.05 mM 1-decanesulfonic acid sodium salt and 12% v/v acetonitrile. The mobile phase was adjusted to pH 6.0 with acetic acid, filtered under vacuum, through a 0.22 μm Nylon membrane filter 47 mm diameter filter (Sigma) and degassed with helium. The column was pumped at a flow rate of 0.1 ml/min at ambient temperature (22–24 °C).

Detection conditions were as follows: guard cell (ESA 5020) = +350 mV, microdialysis cell (ESA 5014B) E1 = −150 mV, E2 = +250 mV, and a recorder range of 50 nA. The detector output was captured and analyzed using Waters Millenium32, version 3.20. The concentrations of NE in each sample were calculated from the integrated chromatographic peak height and expressed as percentage of baseline release, the mean of the first four microdialysate samples.

In vitro recovery of the microdialysis probes was determined as described previously (Lavicky and Dunn, 1995). The recoveries were between 12 and 16% for NE depending on the probe. Because the diffusion of materials from within brain tissue is likely to be different from that in a saline solution (Benveniste, 1989), we did not correct the data for these in vitro recoveries.

2.7. Experimental procedure

Data are presented from a total of 36 runs that yielded a full set of results (i.e., temperature, behavior, NE, ACTH, and corticosterone). Most of the rats were used in two separate runs, with the saline/IL-1 treatment order reversed. Each animal was familiarized with the microdialysis chamber (15 in. animal enclosure, Instech Laboratories, Plymouth Meeting, PA)