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Effects of interleukin-1 beta injections into the subfornical organ and median preoptic nucleus on sodium appetite, blood pressure and body temperature of sodium-depleted rats

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HIGHLIGHTS

- IL-1β and hydrosaline homeostasis
- Inhibition of salt appetite by IL-1β injections into the SFO and MnPO
- Increase in body temperature by IL-1β injections into the SFO and MnPO
- Hypertensive effect of IL-1β injection into the SFO and MnPO

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ABSTRACT

Interleukin-1β (IL-1β) appears to be the mediator of the reciprocal communication between the brain and the immune system. IL-1β has been shown to modulate homeostatic functions including fever, feeding, drinking and cardiovascular control. The aim of the present study was to investigate the effects of IL-1β injections directly into the subfornical organ (SFO) and the median preoptic nucleus (MnPO) on salt appetite, hedonic response, locomotion, body temperature and blood pressure in sodium-depleted rats. IL-1β injections into the SFO and MnPO at the doses of 0.2, 0.4, 0.8 and 1.6 ng/0.2 μl promoted a dose-dependent inhibition of salt intake in sodium-depleted rats. Results of the "dessert" test and the "open field" test suggested that the inhibition of salt appetite is not due to any changes in the hedonic aspect of ingestive behavior or to changes in locomotor activity. As expected, IL-1β injections into the SFO and MnPO promoted an increase in body temperature. However, the fever induced by IL-1β injected into the SFO was slower than the increase in body temperature obtained following IL-1β injection into the MnPO. Furthermore, IL-1β at a dose of 1.6 ng/0.2 μl directly injected into the MnPO led to a significant increase in blood pressure, while injection of the same concentration of IL-1β into the SFO caused no significant change in blood pressure or heart rate. The action of pro-inflammatory cytokines may interfere with the normal control of body temperature, blood pressure and fluid homeostasis, producing the adjustment required to cope with infection and inflammation. Further studies are required to clarify the mechanisms involved in fever, blood pressure increase and inhibition of sodium appetite induced by injections of IL-1β into the SFO and MnPO in sodium-depleted rats.

1. Introduction

The interaction between the immune and central nervous systems promotes behavioral, neuroendocrine and autonomic adjustment to cope with infection, injury and cancer [1,2]. The pro-inflammatory cytokines are the mediators of this communication. Cytokines produced in the periphery may reach the brain by neural and humoral pathways, and may involve toll-like receptors present in cells of the circumventricular organs and endothelial cells of the choroid plexus. Another mechanism is by specific cytokine transporters at the blood-brain barrier [2–6]. The crosstalk between these systems permits the brain to monitor the peripheral innate immune responses and act in parallel in disease conditions, correcting the homeostatic functions.

The set of behavioral responses observed in sick individuals during activation of the immune system includes lethargy, anhedonia, altered

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sleep patterns, depression, anorexia and drinking inhibition, and is designated "sickness behavior". On the other hand, behavioral stimuli such as stress, overeating and sleep may influence the immune response [7–12]. It has been suggested that IL-1β within the brain may mediate the sickness behavior induced by lipopolysaccharide (LPS) and cytokine injections in the periphery.

The intraperitoneal injection of LPS triggers an inflammatory response and increases Fos expression and IL-1β mRNA in the brain, initially in perivascular cells, meningeal cells and neurons in circumventricular organs such as the subfornical organ (SFO), organ vasculosum of the lamina terminalis (OVLT) and area postrema, and afterwards within the brain parenchyma areas where the blood-brain barrier is intact [5,13,14]. LPS then activates different brain areas including the median preoptic nucleus (MnPO), paraventricular nucleus (PVN) and amygdala (AMY) [13,15–19]. Cytokines may also be produced by neuronal and glial cells in the brain and may modulate the release of neurotransmitters and hormones [1,2].

Anorexia and adipsia are observed as part of the sickness behavior following LPS and IL-1β injections, either when given into the periphery or into the brain [20–25]. However, relatively few studies have addressed the effect of LPS or cytokines on salt appetite. Peripheral injection of LPS and intracerebroventricular administration of IL-1β have been shown to decrease salt intake in sodium-depleted rats [23,26,27].

A complex brain network of inhibitory and stimulatory inputs controls water intake and salt appetite [28–35]. Both the SFO and the MnPO are involved in the control of water and salt intake and both seem to be important for the crosstalk between the immune and brain systems [36–41].

Based on data in the literature, it appears reasonable to speculate that the increase in IL-1β levels in the periphery and in the brain promotes behavioral and autonomic changes, allowing the individual to cope with infection, inflammation and injury. The aim of the present study was to investigate the effects of IL-1β injections into the SFO and the MnPO on salt appetite, hedonic responses, locomotion, body temperature and blood pressure.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–280 g) were kept under controlled light (lights on from 6 am to 7 pm) and temperature (22–24 °C) conditions with free access to tap water and laboratory chow (Biobase Alimentação Animal, Santa Catarina, Brazil). All experiments were conducted between 7 and 11 a.m. Groups of rats used in one experimental set were not reused in any other part of the study. The experimental protocols complied with the recommendations of the National Institutes of Health (USA) and were approved by the Institution's Animal Ethics Committee (CEUA-ICS-UFBA # 056/2014).

2.2. Surgical procedures

The animals were anesthetized (ketamine/xylazine; 80/7 mg/kg i.p.) and placed in the stereotaxic apparatus with the skull leveled between the bregma and lambda. The guide cannulas (22-gauge) were implanted either into the SFO (anteroposterior = 0.9 mm behind the bregma; lateral = 0 mm; vertical = 4.8 mm below the skull) or into the MnPO (anteroposterior = 0.3 mm behind the bregma; lateral = 0 mm; vertical = 5.8 mm below the skull). The tips of the guide cannulas were positioned at a point 1 mm above the MnPO or SFO. These coordinates were based on The Rat Brain Atlas by Paxinos and Watson [42]. The guide cannula was fixed to the skull with metal screws and dental cement and an obturator was provided to avoid obstruction. Four days after implantation of the guide cannulas, two groups of animals bearing cannulas in the SFO and two groups with cannulas in the MnPO were submitted to a second surgical procedure in which a catheter (PE50) filled with heparin solution (1000 U/ml) was inserted into the left carotid artery and exteriorized at the nape of the animal's neck to permit blood pressure recording. This procedure was also performed under ketamine/xylazine (80/7 mg/kg i.p.) anesthesia. After surgery, the animals were treated with an antibiotic combination of penicillin and streptomycin (Pentabiótico, Fort Dodge Ltda., Brazil; 0.2 ml/rat i.m.) and with the analgesic/anti-inflammatory agent, flunixin meglumine (2.5 mg/kg i.m.). The animals were then housed in individual cages, with free access to laboratory chow, distilled water and 1.5% saline solution, and handled every day to minimize the stress of the experimental procedure.

2.3. Histological procedures

At the end of the experiments, the animals were anesthetized with ketamine/xylazine (80/7 mg/kg i.p.) and given injections of 2% Evans Blue dye into the SFO or MnPO at a volume of 200 nl. Five minutes later they were submitted to transcardiac perfusion with isotonic saline solution followed by 10% formalin. The brains were then removed and post-fixed in 10% formalin for 24 h at 4 °C. After that, the brains were transferred to a 30% sucrose solution and maintained at 4 °C for at least another 72 h for cryoprotection before being sliced (40 μm) in the cryostat. To confirm the injection sites, the slices were stained with cresyl violet and analyzed by light microscopy.

2.4. Drugs and microinjections

Recombinant human interleukin-1β (rhIL-1β, *Escherichia coli* derived; Sigma Co., St. Louis, MO, USA) was dissolved in sterile isotonic saline solution. Central injections were given using a Hamilton microsyringe connected through polyethylene tubing to a 30-gauge injector that was 1 mm longer than the guide cannula. A total volume of 200 nl was slowly injected (60 s), with the injector remaining in the guide cannula for an additional 60 s. The doses of IL-1β (0.2, 0.4, 0.8 and 1.6 ng/rat) used were based on previous studies conducted by our group and on data published in the literature [25,43–46]. Furosemide (Sanofi-Aventis Famacêutica Ltda, São Paulo, Brazil), a loop diuretic, was injected subcutaneously at the dose of 20 mg/kg.

2.5. Experimental protocols

2.5.1. Sodium depletion

To induce sodium depletion, the rats received a subcutaneous injection of furosemide (20 mg/kg) 24 h prior to the experimental sessions. The animals had access to distilled water, 1.5% saline solution and standard rat chow from the moment immediately following implantation of the guide cannula into the SFO or MnPO until the time of furosemide administration. Access to 1.5% saline ceased immediately after the furosemide injection and from that moment on the animals continued to have free access to distilled water, while normal rat chow was replaced by a low sodium diet (0.001% $Na+$ and 0.33% $K+$). Control animals not submitted to sodium depletion received subcutaneous injections of isotonic saline solution instead of furosemide. Different groups of sodium-depleted rats received injections of IL-1β (0.4, 0.8 and 1.6 ng/0.2 μl) or isotonic saline solution into the SFO or MnPO, and the effect on water and salt intake was evaluated. The bottles containing 1.5% saline solution and distilled water were reintroduced into the cages 15 min after central injections. Fluid intake was recorded at 5, 15, 30, 45, 60, 90 and 120 min after reintroduction of the bottles into the cages.

2.5.2. Dessert test

To investigate whether the central administration of IL-1β was able to modify salt intake through a non-specific, general inhibition of ingestive behavior, a well-established model of hedonic behavior in rats was used [47,48]. In this experiment, after implantation of the guide cannula into the SFO or MnPO, the animals were kept in their usual individual