

Systemic Interleukin-1 β stimulates the simultaneous release of norepinephrine in the paraventricular nucleus and the median eminence

Sheba M.J. MohanKumar*, P.S. MohanKumar

Neuroendocrine Research Laboratory, Department of Pathobiology and Diagnostic Investigation, Veterinary Medical Center, A-20, Michigan State University, East Lansing, MI 48824, USA

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Abstract

Interleukin-1 β (IL-1 β), a cytokine with pronounced central effects such as fever, anorexia, analgesia, etc., is also known to activate the hypothalamo–pituitary–adrenal (HPA) axis. Corticotropin releasing hormone (CRH) neurons located in the hypothalamus are important for HPA activation. The cell bodies of CRH neurons are located in the paraventricular nucleus (PVN) and their terminals are present in the median eminence (ME). Although the catecholamines, norepinephrine (NE) and dopamine (DA) are believed to be crucial factors in the stimulation of CRH neurons, it is not clear if they affect the cell bodies or terminals of these neurons to cause HPA activation. This study was done to determine if IL-1 β affects NE and DA release at the level of CRH cell bodies or their terminals. Adult male Sprague–Dawley rats were implanted with two push–pull cannulae, one in the PVN and another in the ME, and were subjected to push–pull perfusion. They were treated either with 0, 1 or 5 μ g of IL-1 β . Perfusates were collected for 2 h after treatment and analyzed for NE concentrations using HPLC-EC. NE levels in the control and low dose groups did not change significantly during the entire period of observation both in the PVN and ME. In contrast, treatment with 5 μ g of IL-1 β produced a marked increase in NE release in the PVN at 20 and 40 min post-treatment. NE release in the ME increased from 10 to 140 min post-treatment. There were no significant changes in the release of DA from both these areas. These results indicate that IL-1 β increases NE levels both in the PVN and in the ME and this could be a possible mechanism by which it stimulates the HPA axis.

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1. Introduction

Interleukin-1 β (IL-1 β) is a cytokine that has a number of central effects such as fever, anorexia, analgesia, etc. [7]. One of the most important actions involving the neuroendocrine system is its stimulatory effect on the hypothalamo–pituitary–adrenal (HPA) axis [25]. The secretion of corticosterone that is the ultimate end point of HPA activation is brought about by an increase in the secretion of corticotropin releasing hormone (CRH) from the hypothalamus. CRH acts on corticotrophs in the anterior pituitary to increase the secretion of Adrenocorticotrophic hormone

(ACTH) which acts on the adrenal cortex to increase corticosterone [21]. The CRH neurons that are responsible for HPA activation are concentrated in the paraventricular nucleus (PVN) of the hypothalamus, with their terminals located in the median eminence (ME) [5]. Among the various neurotransmitters that can influence these neurons, the catecholamines, norepinephrine (NE) and dopamine (DA) are believed to be crucial factors [21].

NE is believed to stimulate CRH secretion [21]. Injections of NE into the PVN are known to cause an increase in HPA activation [13]. The PVN also receives rich noradrenergic innervation from the brain stem [21] and lesioning of the ventral noradrenergic bundle (VNAB) that carries noradrenergic fibers to the hypothalamus has caused a reduction in ACTH secretion [9]. Moreover, usage of noradrenergic depletors has

* Corresponding author. Tel.: +1 517 432 2876; fax: +1 517 432 7480.
E-mail address: mohankumrs@cvm.msu.edu (S.M.J. MohanKumar).

caused marked decreases in corticosterone secretion [2]. All these studies prove that NE is critical for HPA activation. In contrast to NE, the role of DA is controversial [21].

IL-1 most likely affects CRH neurons in the hypothalamus to stimulate the HPA axis. The exact mechanism by which IL-1 does this is unclear. Since CRH perikarya are concentrated within the PVN of the hypothalamus, there is a good possibility that IL-1 may act at the level of this nucleus. In fact, IL-1 receptors have been identified in endothelial cells within the PVN [12]. On the other hand, IL-1 could also act at the level of CRH terminals to stimulate the secretion of this hormone. But it is not clear if NE is needed to stimulate CRH perikarya in the PVN or to stimulate CRH release from nerve terminals located in the median eminence [14]. Previously, we have shown that IL-1 β can stimulate the release of NE within the PVN [17]. However, it is not clear if IL-1 can influence NE activity both at the level of CRH cell bodies and terminals simultaneously to stimulate CRH secretion. This study was, therefore, performed using a novel technique that involved the implantation of two cannulae, one in the PVN and the other in the ME. Push–pull perfusion was performed at both sites simultaneously to measure the effect of IL-1 on NE release in these two areas.

2. Methods

2.1. Animals

Adult male Sprague–Dawley rats (3–4 months old, Harlan Sprague–Dawley, Inc., Indianapolis, IN) were used in the experiments. They were housed in light-controlled (lights on from 05:00 to 19:00 h) and air-conditioned ($23 \pm 1^\circ\text{C}$) animal rooms and were given rat chow and water ad libitum.

2.2. Push–pull cannula implantation

The animals were weighed and randomly divided into three groups of eight animals each. Prior to surgery, the animals were given atropine sulphate (2.2 mg/kg, i.p.) to reduce salivary secretions and to keep the airways open and were anesthetized using sodium pentobarbital (50 mg/kg of BW, i.p.). The rats were implanted with push–pull cannulae in the PVN and the ME using a stereotaxic apparatus (Kopf, Tujunga, CA). Construction of the push–pull cannula has been described previously [16]. It consisted of a 8.5 mm-long outer cannula made from a 22-gauge hypodermic needle. The coordinates used for implantation of the cannula in the PVN were 8.2 mm ventral, 1.8 mm posterior and 0.2 mm lateral to the Bregma [24]. The cannula to the ME was 14 mm long, and was inserted at an angle of 20° through a hole drilled 8.8 mm posterior to the Bregma. The cannulae were secured in place with screws and dental cement. After implantation, 29 G stainless steel stylets were introduced into the cannulae to prevent blockade by gliosis. Rats were housed individually in flat-bottomed cages after surgery. They were periodically

monitored for signs of infection or discomfort and were used in the experiments 10 days later. Rats that showed any sign of infection were excluded from the study. All procedures involving animals were approved by the Institutional Animal Care and Use Committee.

2.3. Push–pull perfusion procedure

Push–pull perfusion was performed as described earlier [17–19]. On the day of perfusion, the stylet was replaced with an inner cannula assembly, which consisted of two 29-gauge stainless steel tubes of unequal lengths. The longer tube, which protruded 0.5 mm beyond the outer cannula, was used to introduce (push) the perfusion medium at the implantation site. The shorter tube was used to collect (pull) perfusate from the implantation site. The two tubes were kept together in a 2 mm-long piece of Silastic tubing which was mounted with epoxy resin in the lower part of a tuberculin syringe cut at the 0.05 mL mark. The push and pull tubes were connected to two identically calibrated peristaltic pumps (Pharmacia, Uppsala, Sweden). Before starting the perfusion, care was taken to ascertain that the pumps were perfectly balanced. Artificial cerebrospinal fluid (ACSF) was used as the perfusion medium. It consisted of CaCl_2 (0.087 g/L), NaCl (7.188 g/L), KCl (0.358 g/L), MgSO_4 (0.296 g/L) and Na_2HPO_4 (1.703 g/L) and had a pH 7.3. Pump speeds were adjusted to achieve a flow rate of 10 $\mu\text{L}/\text{min}$.

The rats were introduced into the perfusion cages at 08:00 h and perfusion was started at 09:00 h. After collecting two pre-treatment samples, animals in the control group ($n=5$) were injected i.p. with 250 μL of phosphate buffered saline (PBS)–0.1% bovine serum albumin (BSA) at 10:00 h while animals in the experimental groups ($n=4-5$) were treated with either 1 or 5 μg of human recombinant IL-1 β (a kind gift of Dr. Michael Widmer, Immunex Corp.) in 250 μL of PBS–0.1% BSA. Push–pull perfusates were collected from 09:30 to 12:00 h at 10-min intervals at the rate of 10 $\mu\text{L}/\text{min}$. Perfusates were mixed with 0.5 M HClO_4 at the rate of 25:1 (v/v) and stored at -70°C until HPLC analysis. Only rats that had perfusates collected simultaneously from the PVN and ME were included in the study resulting in a N of 4–5 per group.

2.4. Histology

At the end of the observation period, the animals were sacrificed, and their brains were removed and frozen. Serial brain sections (40 μm) of the brain were obtained using a cryostat (Slee, London, UK) maintained at -10°C . The perfusion site was determined by examination of brain sections stained with cresyl violet.

2.5. HPLC-EC

The HPLC-EC system has been described before [17–19]. Briefly, it consisted of a Shimadzu LC-6A pump (Shimadzu,

Columbia, MD) connected to a Sepstik column attached in parallel to a phase II, 5 μm ODS reverse phase, C-18, 76 mm \times 3.5 mm column (BAS, West Lafayette, IN) through a flow splitter assembly. Flow from the Sepstik column passed through a glassy carbon working electrode connected to a LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN), and a C-R6A Chromatopac integrator (Shimadzu, Columbia, MD). The mobile phase consisted of monochloroacetic acid (14.5 g/L), sodium hydroxide (4.675 g/L), octanesulfonic acid disodium salt (0.3 g/L), ethylenediaminetetraacetic acid (0.25 g/L) and acetonitrile (35 mL/L) in pyrogen-free, degassed water, filtered through a Milli-Q purification system (Millipore Co., Bedford, MA). The pH of the mobile phase was adjusted to 3.1 using NaOH. The flow rate of the pump was 1.1 mL/min. The columns and the working electrode were kept in a Shimadzu CTO-6A oven at a temperature of 37 $^{\circ}\text{C}$. The potential of the working electrode was 0.65 V and the range of the detector was 1.0 nA full scale. The sensitivity of the system was <1 pg. At the time of HPLC-EC analysis, the samples were thawed at 60 $^{\circ}\text{C}$ for 1 min. A mixture of 7.5 μL of the sample and 2.5 μL of the internal standard (0.05 M isoproterenol) was injected into the HPLC system. Neurotransmitter release was expressed as pg/min.

2.6. Statistical analysis

The differences in the profiles of NE and DA release in the PVN and ME during the entire observation period were analyzed using repeated measures ANOVA followed by posthoc Fisher's LSD test.

3. Results

3.1. Location of the push–pull cannulae

Fig. 1 depicts the locations of the push–pull cannulae in the three groups of animals. Histological examination revealed

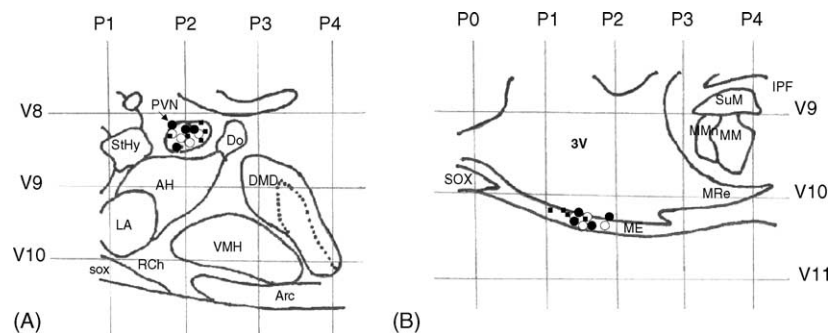


Fig. 1. Location of the push–pull cannula in control (○) and IL-treated (●) 1 μg , (■) 5 μg groups. All cannulae were located in the PVN and the ME. (A) Cannula locations in the PVN. AH, anterior hypothalamic area; Arc, arcuate nucleus; DMD, dorsomedial hypothalamic nucleus diffuse; Do, dorsal hypothalamic nucleus; LA, lateral hypothalamic area, PVN, paraventricular hypothalamic nucleus; RCh, retrochiasmatic area; StHy, striohypothalamic nucleus; VMH, ventromedial hypothalamic nucleus. (B) Cannula locations in the ME. IPF, interpeduncular fossa; Mmm, medial mammillary nucleus; MRe, mammillary recess; Sox, supraoptic decussation; Sum, supramammillary nucleus. P1–4 indicates mm posterior to Bregma. V8–11 indicates mm ventral.

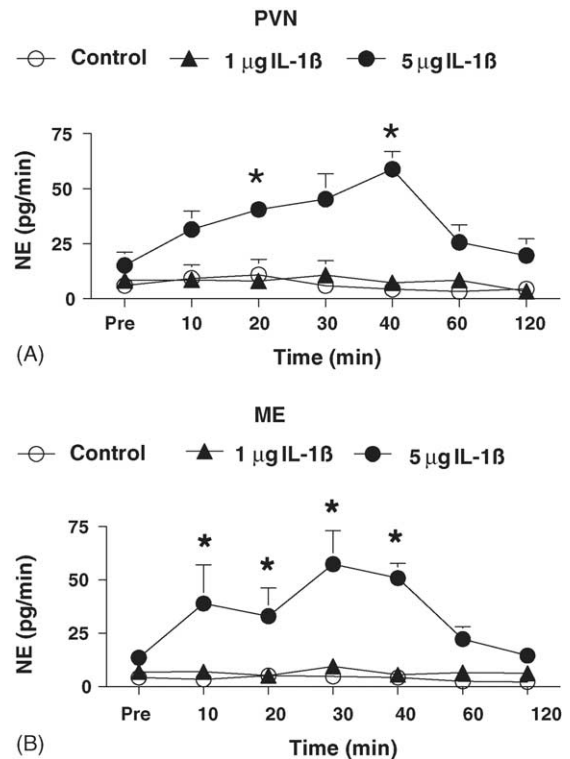


Fig. 2. (A) NE release profile in the PVN. (B) NE release profile in the ME. Push–pull perfusion was performed in these two areas for 2 h following the vehicle or IL-1 injections i.p. (*) Significantly different from the pre-treatment levels.

that the tips of the push–pull cannulae in all the animals were in the PVN and the ME.

3.2. NE release

Profiles of NE release in the PVN after the various treatments are shown in Fig. 2A. Pre-treatment NE release was not different between the three groups. NE release (mean \pm S.E., pg/min) in control animals was 6.03 ± 3.1 prior to treatment and did not change significantly after injection of the vehi-

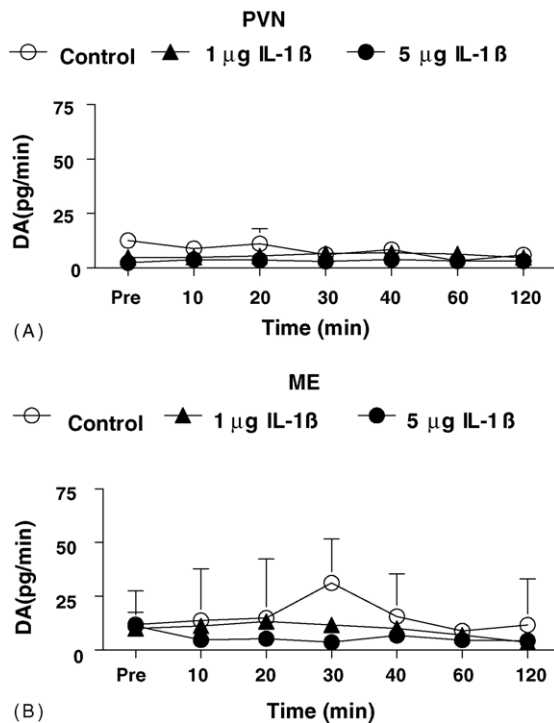


Fig. 3. DA release profiles in the PVN and ME. DA was measured in push–pull perfusates collected over a period of 2 h following the vehicle or IL-1 β injection i.p. (A) DA release profile in the PVN. (B) DA release profile in the ME.

cle. Treatment with 1 μ g of IL-1 β did not affect NE release in the PVN either. In contrast, treatment with 5 μ g of IL-1 β produced a 260% increase (40.6 ± 1.85 compared to 15.2 ± 5.97 pre-treatment) in NE release by 20 min, increased by 380% (58.9 ± 8.01) at 40 min and declined to 25.6 ± 7.98 by 60 min and remained at that level at 120 min (19.6 ± 7.63). In the ME, NE release remained unaffected in the control and 1 μ g groups (Fig. 2B). Treatment with 5 μ g of IL-1 β increased NE release by 280% within the first 10 min and kept it elevated at 20 min (32.9 ± 13.18), increased it further to 57.4 ± 15.75 by 30 min, and was 50.8 ± 8.01 at 40 min before declining to 22.2 ± 5.8 at 60 min. The increases at 30 and 40 min were 425 and 375%, respectively. NE release dropped to pre-treatment levels at 120 min.

3.3. DA release

Unlike NE, DA release in both the PVN and ME remained unchanged during the entire observation period after IL-1 β treatment (Fig. 3).

4. Discussion

Results from the present study show that systemic IL-1 β is capable of stimulating NE release simultaneously in both the PVN and the ME. NE release in the PVN increased within the first 20 min after IL-1 treatment; however, the effect subsided

by 60 min. Similar changes were observed with NE release in the ME. This parallels the profile of ACTH secretion observed in other studies after immune challenge [15].

Perfusing two hypothalamic areas simultaneously in vivo is a novel yet tedious technique. There are only two other reported studies that have used this technique to study the effect of IL-1 on the PVN and ME [31,32]. These studies found that IL-1 increased the secretion of corticotrophin releasing hormone (CRH) in both the PVN and ME prior to the increase seen in plasma ACTH when IL-1 was given icv or iv. The present study takes these results further by showing a similar temporal profile in NE release in these two areas suggesting the possibility that IL-1 affects noradrenergic activity in both these areas simultaneously to affect the HPA axis.

The initial effects of IL-1 on CRH neurons in the PVN were reported in 1990 [26]. Later, IL-1 was found to increase c-fos levels in three subsets of CRH neurons within the PVN [34]. Direct injection of IL-1 into the PVN caused fever production and anorexic effects, reduction in locomotion, body weight loss, etc. [1]. More recently, IL-1 was reported to cause depolarization of PVN parvocellular neurons using whole cell patch clamp recordings [8]. Moreover, the PVN and ME are known to contain IL immunoreactive fibers under normal physiological conditions [33]. Taken together, these studies indicate that IL-1 is capable of acting on the PVN and ME.

There are several lines of evidence to indicate the mechanism by which IL-1 stimulates the HPA axis by acting on these two nuclei. Neurotransmitters such as NE and DA are crucial for the activation of CRH neurons in the PVN [21]. Previously we have provided evidence using push–pull perfusion that IL-1 is capable of stimulating NE release in the PVN [17]. In addition, concentration studies have shown increases in NE in both the PVN and ME in a dose dependant manner after IL treatment that was reversed by IL-1 receptor antagonist [20]. Besides direct evidence for increases in NE levels in the PVN and ME there are other sources of indirect evidence demonstrating the importance of NE in the IL-induced activation of the HPA axis. Lesioning of the dorsal, central or ventral part of the VNAB that carries the majority of noradrenergic fibers from brain stem nuclei to the hypothalamus has caused a marked attenuation of the IL-1-induced ACTH secretion [3]. Also lesioning of the VNAB using 6-hydroxydopamine decreased CRH mRNA expression and reduced the increase in ACTH levels observed after IL-1 treatment [23]. Similarly, treatment with 6-hydroxydopamine abolished IL-1 induced elevations in c-fos in the PVN. This effect was blocked by pre-treatment with desmethyl imipramine that blocks NE depletion indicating that IL-1-induced activation of the PVN is mediated through NE [27].

All these reports support the results from the present study in which we see increases in NE release in the PVN and ME after IL treatment. The timing of NE release in the PVN is, however, different from another reported study [28]. In that study, NE release in the PVN occurred in two spurts, an early brief increase followed by a more sustained increase last-

ing from 80 min post-treatment and reaching a maximum at 140 min. In our previous study, however, we observed an increase in NE release within 20 min after administration of IL-1 β that reached a peak by 70 min before declining to basal levels at 90 min post-treatment [17]. In another study by Ishizuka et al. [11], NE levels were measured in the PVN by microdialysis. Here NE levels increased within 20 min but declined only by 180 min. The fact that the animals in this experiment were subjected to isoflurane anesthesia during microdialysis could have been an important contributing factor. The present study takes these a little further by demonstrating an increase in NE release in both the PVN and the ME with more sustained NE release in the ME which contains the terminals of CRH neurons.

Both the PVN and ME receive rich noradrenergic innervation from the brain stem noradrenergic neurons located in the A1, A2 and A6 noradrenergic nuclei. NE cell bodies in the A2 region innervate a majority of medial and parvocellular part of the PVN while A1 neurons innervate the magnocellular part of the PVN [6]. There is evidence to indicate that the ME may receive noradrenergic innervation from the superior cervical ganglion [10] although the majority of the innervation appears to come from the A1 and A2 nuclei [22]. A recent study indicates that the PVN may have downstream connections to the brain stem noradrenergic neurons and that these complex reciprocal relationships between the PVN and the brain stem are likely to be involved during an immune challenge [4]. These may also serve as an ultra-short loop negative feedback mechanism. The vagus and the area postrema may also act as other sites/pathways by which IL-1 activates the HPA axis [11].

Unlike NE, the role of DA in CRH secretion is not very clear. Studies that have seen an increase in NE concentrations in the PVN and ME after IL treatment demonstrate an increase of only DA levels in the PVN [20]. Lesioning with 6-hydroxydopamine that depletes NE levels but does not affect DA levels causes marked decreases in ACTH, CRH and CRH mRNA levels indicating that NE plays a more important role than DA in IL-1 induced activation of the stress axis [23]. The results from the present study supports this further by demonstrating an increase in NE release after IL treatment while DA release remains unaffected.

Besides NE other factors such as nitric oxide [29] and prostaglandins [30] may also be involved in the IL-induced activation of the stress axis. Nevertheless, results from the present study indicate that IL-1 is capable of stimulating NE release simultaneously in the PVN and NE indicating that this may play an important role in the secretion of CRH.

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