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α-MSH and γ-MSH modulate early release of hypothalamic PGE2 and NO induced by IL-1β differently

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Abstract

Interleukin-1β (IL-1β) stimulates corticotropin-releasing hormone (CRH) secretion in hypothalamus, which involves the release of prostaglandins (PGE2) and nitric oxide (NO). We have demonstrated that melanocortins can inhibit the early effects of IL-1β on the HPA axis by acting on the central nervous system (CNS). Our study investigated whether α-melanocyte stimulating hormone (α-MSH) and γ-MSH could inhibit IL-1β-induced PGE2 and NO release in hypothalamus in the rapid activation of the HPA axis. An i.c.v. injection of 12.5 ng/μl of IL-1β significantly increased the release of PGE2 and NOS activity in the hypothalamus. Treatment with α-MSH (0.1 μg/μl) inhibited the effect of IL-1β on PGE2 release. Also, γ-MSH (1 μg/μl) eliminated the increase in NOS activity induced by IL-1β. Our data indicate the modulatory role of melanocortins in the early hypothalamic response to IL-1β, with different regulation of PGE2 and NO release.

Keywords: Prostaglandin; Nitric oxide; Interleukin-1β; Melanocortins; Hypothalamus

Interleukin-1β (IL-1β), an immune mediator, has an important role in immune regulation of the neuroendocrine system, primarily by increasing secretion of the corticotropin-releasing hormone (CRH) from the median eminence as a result of actions on the paraventricular nucleus (PVN) of the hypothalamus [23].

The effects of IL-1β have been linked to activation of cyclooxygenase (COX)-2, resulting in prostaglandin (PG) production, which then acts directly on neurons in the PVN [2]. IL-1 receptor-1 and COX-2 enzyme are colocalised in the PVN [14]; both lipopolysaccharide (LPS) and IL-1β administration increase COX-2 mRNA and protein expression in the brain [4]. Also, the attenuation of the stimulatory effect of IL-1β on CRH secretion in the presence of the nonspecific COX inhibitor, indomethacin [2] indicates that this enzyme mediates the effects of this cytokine. Although COX-2 is an upstream enzyme in the production of several lipid mediators, it is probable that PGE2 could participate specifically by mediating the effects of IL-1β on the PVN [24]. Recently, Ferri and Ferguson demonstrated that the rapid effects of IL-1β on parvocellular neurons in the PVN are dependent on COX-2 activation and subsequent PGE2 production [11]. They also showed that another common inflammatory mediator, nitric oxide (NO), contributes to the IL-1β-induced depolarization of parvocellular neurons [10].

α-Melanocyte stimulating hormone (α-MSH), a melanocortin with potent anti-inflammatory properties, is a potent inhibitor of iNOS induction in cultured macrophages and NO production in liver and microglia [7,9,22]. It also inhibited PGE2 production in brain tissue [26]. α-MSH was recently reported to inhibit iNOS and COX-2 expression in hypothalamus [5]. γ-MSH also attenuates inflammatory responses to endotoxins in vivo and in vitro [6].

Melanocortins affect cells by binding to five different G protein-coupled receptors called melanocortin receptors (MC1-R to MC5-R) [6]. Ligand binding to MCRs activates adenyl cyclase, leading to the production of cAMP and subsequent activation of protein kinase A (PKA). MC3-R activation could also lead to an inositol phospholipid/Ca^{2+}-mediated signaling system

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[6]. The distribution of MC4-R mRNA in the paraventricular nuclei of the hypothalamus is consistent with the proposed roles of the central melanocortin system in neuroendocrine regulation [13]. Also, α-MSH, through MC4-R, inhibits iNOS and COX2 expression induced by LPS in the hypothalamus [5]. Autoradiographic studies show that MC3-R is not predominant in PVN [15,21].

We demonstrated that α-MSH and γ-MSH can inhibit the early effects of IL-1β on the hypothalamic-pituitary-adrenal (HPA) axis, by acting on the central nervous system (CNS) [8]. This effect was observed shortly after injection of the peptides and therefore could not have involved de novo synthesis of NOS and COX2 enzymes. In this study, we tested whether the melanocortin effect is due to an inhibition of IL-1β-induced PGE2 and NO release from hypothalamus, in the early HPA axis activation response.

Male Wistar rats weighing 250–300 g were kept at 21 ± 1 °C, lights on 7:00–19:00 with free access to food and water. Animals were initially housed five per cage, and after surgery individually, in order to avoid damage to the guide cannula. Rats were handled daily for 20 days before the experiments, accustomed to i.c.v. injections after surgery for the entire recovery period. All experiments were performed according to the guidelines of NIH Guide for the Care and Use of Laboratory Animals as approved by the School of Chemical Sciences, National University of Cordoba Animal Care and Use Committee. Every effort was made to minimize pain and discomfort.

Animals anesthetized with ketamine hydrochloride (55 mg/kg) and xylazine (11 mg/kg) were placed in a stereotaxic apparatus for cannula implantation. A stainless steel guide cannula (22-gauge) was implanted in the right lateral ventricle (i.c.v.). The implantation coordinates according to Paxinos and Watson [20] were 0.8 mm posterior to the bregma, 1.8 mm lateral to the midline, and 4.1 mm ventral to the skull surface. The animals were allowed to recover from surgery for a week before performing the experiments. Drugs or vehicle were administered manually i.c.v. via an internal cannula extending 0.5 mm beyond the guide cannula, connected by a flexible tube to a 10 μl Hamilton syringe. Each infusion was done over a period of 1 min. After each injection, the cannula was left in place for 1 min to prevent back flow of injected solution. Total volume of drugs and vehicle was 1 μl.

Rats were randomly assigned to treatment groups. All injections were applied between 10:00 and 11:00 to avoid any circadian variation of PGE or NOS activity. On the test day, animals were injected i.c.v. with vehicle (sterile saline solution), IL-1β (12.5 ng), α-MSH (0.1 μg) or γ-MSH (1 μg). To study the interaction between IL-1β and either α-MSH or γ-MSH, peptides were administered 15 min after IL-1β. Doses were those determined in a previous study [8]. Animals receiving saline solution injections were the control group. To avoid any effect of variations in the number of injections, all animals received two injections administered 15 min apart. When IL-1β or peptides were tested alone, another injection of vehicle was given. Thirty minutes after the first injection the animals were killed by decapitation. The brains were rapidly removed, placed on ice and the hypothalami dissected to measure PGE release or NOS activity. To measure PGE release, the hypothalamus was cut at the midline into two symmetrical parts, which were then washed in Krebs-Ringer bicarbonate (KRB) buffered at pH 7.4 and supplemented with 0.1% bovine serum albumin (BSA) and 0.2% glucose. Tissues were placed in a tube with 0.5 ml KRB containing the same drugs that animals received in vivo, in order to maintain conditions similar to those in vivo throughout the incubation period. The concentrations used were: (a) IL-1β (12.5 ng/ml); (b) α-MSH (0.1 μg/ml); (c) γ-MSH (1 μg/ml); (d) IL-1β (12.5 ng/ml) and γ-MSH (1 μg/ml); and (e) IL-1β (12.5 ng/ml) and α-MSH (0.1 μg/ml). Tissues were incubated in a shaker (40 cycles per minute) at 37 °C in an atmosphere of 95% 02/5% CO2 for 30 min. Medium samples were stored at −70 °C until assayed for PGE. Tissue was homogenized in KRB and stored at −20 °C. Proteins were determined with an aliquot of homogenate by the Lowry method [16]. PGE2 was determined with an Elisa kit according to the manufacturer's instructions (Assay Designs, USA).

NOS activity in hypothalami was determined by measuring L-[U-14C]arginine conversion to [U-14C]citrulline as described by Bredt et al. [1]. Briefly, one hypothalamus per vial was preincubated for 8 min in KRB as described above for PGE determinations. Then, hypothalami were homogenized in 500 μl of 20 mM Hepes (pH 7.4), 1 mM dithiothreitol (DTT), 0.45 mM CaCl2 and 400 μM NADPH. We incubated 450 μl with 0.1 μCi L-[U-14C]arginine (Amersham Pharmacia Biotech, Buckinghamshire, UK, 300 mCi/mmol) for 30 min more as described above. Then the tubes were centrifuged at 10,000 rpm at 4 °C for 10 min. We immediately applied 400 μl of supernatants to individual columns of Dowex AG 50W-X8 200–400-mesh sodium form (Bio-Rad), previously stabilized with 20 mM Hepes (pH 7.4) in order to separate [14C]citrulline in the supernatants, then washed with 3 ml double distilled water. [14C]citrulline in the eluent was quantified by liquid scintillation using a β-counter scintillation counter. Before the L-[14C]arginine was added to homogenized hypothalamus, 20 μl were separated for protein determination by the Lowry method [16].

All data are expressed as the mean ± S.E.M. NOS activity is a percentage of control values, where vehicle-injected levels are 100%. All data were analyzed by one-way ANOVA. When ANOVA was significant ($p < 0.05$), post-hoc comparisons were run with the least significance difference (L.S.D.) test.

i.c.v. injection of IL-1β (12.5 ng/μl) significantly increased release of PGE2 from incubated hypothalamus and NOS activity in homogenized hypothalamus. This increase in PGE2 release occurred quite soon after IL-1β injection and was about 60% greater than the control. Mean basal PGE2 release was about 0.06 (pg/mg protein). i.c.v. injection of α-MSH after IL-1β inhibited the effect of IL-1β on PGE2 release, the combined treatment producing no significant changes in PGE2 release compared to the control; also, PGE2 release was statistically different from the group that received IL-1β alone. Treatment with α-MSH alone produced no significant changes in PGE2 release (Fig. 1). γ-MSH per se produced no significant changes in PGE2 release compared with control or IL1β treated animals. γ-MSH was not able to attenuate the increase induced by IL-

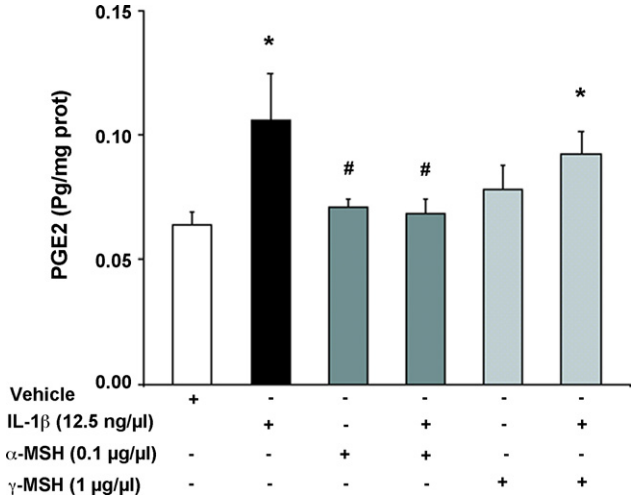

Fig. 1. Effect of melanocortins on IL-1β-induce PGE2 release. PGE2 release from hypothalamic explants was measured after i.c.v. administration of vehicle, IL-1β (12.5 ng/μl) or α-MSH (0.1 μg/μl) or γ-MSH (1 μg/μl) or each melanocortin 15 min after the injection of IL-1β. $n = 6$ animals per group. $^{*}p < 0.05$ vs. control, $^{\#}p < 0.05$ vs. IL-1β.

1β administration (γ-MSH versus γ-MSH + IL1β $p = 0.2350$) (Fig. 1).

i.c.v. injection of IL-1β (12.5 ng/μl) produced a rapid increase in NOS activity, about 13% higher than in animals administered vehicle (Fig. 2). This increase was eliminated by treatment with γ-MSH (1 μg/μl) after IL-1β. However, administration of α-MSH (0.1 μg/μl) was unable to inhibit the effect of the cytokine in animals treated with this combination. The percentage of change respect to control animals after the treatment with α-MSH + IL-1β is statistically different with respect to the control but not with respect to IL-1β alone (α-MSH + IL-1β versus IL-1β $p = 0.8480$).

When L-NAME, an inhibitor of NOS activity, was added to the hypothalamic homogenate, activity of the enzyme was

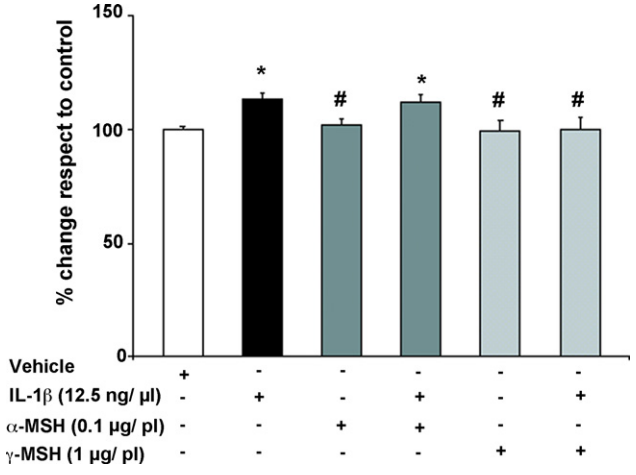

Fig. 2. Effect of melanocortins on IL-1β-induced NOS activity. NOS activity in homogenized hypothalami was measured 30 min after administration of vehicle, IL-1β (12.5 ng/μl) or α-MSH (0.1 μg/μl) or γ-MSH (1 μg/μl) or each melanocortin 15 min after the injection of IL-1β. Changes are expressed as percentages (±S.E.M.) vs. control levels. $n = 5$–7 animals per group. $^{*}p < 0.05$ vs. control, $^{\#}p < 0.05$ vs. IL-1β.

almost completely inhibited (data not shown). Treatment with the melanocortins α-MSH or γ-MSH injected alone produced no changes in NOS activity (Fig. 2).

Our results demonstrate that α-MSH and γ-MSH are able to inhibit PGE2 and NO release induced by i.c.v injection of IL-1β. Thus, α-MSH can modulate PGE2 release whereas γ-MSH can modify NO release. Although all natural melanocortin peptides show the same order of affinity, γ-MSH has the strongest affinity for MC3R among melanocortin receptors [27]. Thus, the inhibitory effect of γ-MSH on NO release could be mediated by MC3R.

Since these effects were observed very shortly after IL-1β treatment, they could not have involved enzyme synthesis [24].

Recent data suggest that physiological effects of PGE2 can occur within minutes in hypothalamic slices. Small amounts of this prostanoid may be secreted rapidly via IL-1β-activation of constitutively expressed COX-2 [4]. Thus, IL-1β triggers rapid production of PGE2, this response being essential for CRH release by PVN [11]. It has been suggested that production of PGE2 released in the brain occurs via two pathways: (a) by fast transient production dependent on activation, possibly through MAPK p38 phosphorylation of available constitutive PGE2 synthesis enzymes, independent of transcription and NF-κB activation; and (b) by slow, sustained PGE2 synthesis involving induction of synthesis of NF-κB-mediated enzymes [24].

Although these effects appear to occur within a short time frame, previous studies have not investigated the modulatory effect of melanocortins on IL-1β-stimulated PGE2 release from the PVN during this period. In other reports α-MSH was found to inhibit PGE2 synthesis in brain tissue [3,26]. α-MSH also attenuated the stimulatory action of LPS on COX2 expression. These effects were observed several hours after peripheral LPS administration. Testing with a selective melanocortin receptor antagonist suggests that α-MSH may exert this effect by activating central MC4-R [5].

Our results demonstrate that IL-1β induced increased PGE2 release from hypothalamic explants after i.c.v. injection in an ex vivo experimental model. These results concord with previously cited reports that IL-1β acts locally in hypothalamus and produces PGE2 release after brief exposition to an immune challenge (11, 24). α-MSH inhibition of the stimulatory effect of IL-1β on PGE2 release could have been mediated by MC4-R, since the MC4R, unlike MC3R, is found in both the parvocellular and magnocellular of the PVN of the hypothalamus, suggesting a role in regulation of activity of the HPA axis via vasopressinergic and corticotropic neurons [17].

α-MSH was shown to inhibit activation of p38 MAPK in different experimental models. For example, α-MSH inhibited LPS-induced activation of p38 kinase in leukocytes [28]. The melanocortin analog NDP-MSH also inhibits p38MAPK activation and cytokine overexpression in the brain, after transient forebrain ischemia [12]. Therefore, it could be suggested that the suppressant effect of α-MSH on PGE2 release may be caused by inhibition of the p38 MAPK, necessary for phosphorylation of cPLA and other enzymes involved in the pathways enabling rapid PGE2 production.

Also, many studies have demonstrated that IL-1β causes both PGE2 and NO release. Uribe et al. have shown that LPS injection induced a rapid, short-lived increase of NO concentration in PVN corresponding to the initiation of ACTH response [25].

Our results showed that γ-MSH, an MC3-R agonist, is able to inhibit IL-1β-induced NO production, while α-MSH was ineffective. These results concord with reports demonstrating that central injection of β-MSH, γ1-MSH and γ2-MSH reduced LPS-induced increase in brain NO in mice, while α-MSH was less effective [18]. Moreover, a recent report demonstrated that MC3-R is the principal MCR involved in mediating inhibition of NO production during brain inflammation [19]. We have previously demonstrated that the i.c.v. injection of IL-1β, in the same dose used in the present work, induced a rapid activation of HPA axis [8]. This effect was inhibited by α-MSH or γ-MSH, also in the same doses tested here [8]. The present results demonstrate that γ-MSH could modulate NO production. Considering our previous results in vivo, it is tempting to assume that the change in NO production induced by IL-1β is biologically relevant and also its inhibition by γ-MSH.

Our results suggest that the anti-inflammatory effects of α-MSH and γ-MSH could involve activation of the melanocortin receptors MC4-R and MC3-R, respectively. Therefore, the activation of different signaling pathways could result in modulation of the production of PGE2 and NO induced by IL-1β in the brain, in early HPA axis activation.

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