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INTERACTION BETWEEN GABA AND NOREPINEPHRINE IN INTERLEUKIN-1 β -INDUCED SUPPRESSION OF THE LUTEINIZING HORMONE SURGE

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Abstract

Interleukin-1 β (IL-1 β), a cytokine that is closely associated with inflammation and immune stress, is known to interfere with reproductive functions. Earlier studies have demonstrated that IL-1 β inhibits the luteinizing hormone (LH) surge during the afternoon of proestrus in female rats. We have shown that this effect is most probably mediated through a reduction in norepinephrine (NE) levels in the medial preoptic area (MPA) of the hypothalamus. However, the mechanism by which IL-1 β decreases NE levels in the MPA is unclear. We hypothesized that the inhibitory neurotransmitter, GABA could play a role in decreasing NE levels in the MPA. To test this, ovariectomized, steroid-primed rats were injected (i.p.) with either PBS-BSA (control) or 5 μ g of IL-1 β , alone or in combination with i.c.v. administration of GABA-A and GABA-B receptor antagonists, Bicuculline and CGP 35348 (CGP) respectively. Animals were subjected to push-pull perfusion of the MPA and perfusates collected at 30 min intervals were analyzed for both NE and GABA levels using HPLC-EC. Simultaneously, serial plasma samples were obtained through jugular catheters and were analyzed for LH levels using RIA. Compared to control rats, NE levels decreased significantly in the MPA in IL-1 β -treated rats ($p < 0.05$). Concurrently, there was a significant increase in GABA levels in the MPA ($p < 0.05$). The GABA-A receptor antagonist, bicuculline, was able to reverse the effect of IL-1 β on NE and LH, while the GABA-B receptor antagonist, CGP 35348 was without any effect. This leads us to conclude that the IL-1 β -induced suppression of the LH surge is most probably mediated through an increase in GABA levels in the MPA which causes a reduction in NE levels. This is probably one of the mechanisms by which IL-1 β inhibits reproductive functions.

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Keywords

GABA; Norepinephrine; Luteinizing hormone; Interleukin-1 β ; push-pull perfusion; GABA antagonists

Introduction

The cytokine, interleukin-1 β (IL-1 β) is known to inhibit reproductive functions (Kalra et al., 1990; Rivest and Rivier, 1993a; Rivier and Rivest, 1991; Rivier and Vale, 1990). It does this by affecting various parts of the reproductive axis or the hypothalamo-pituitary-gonadal (HPG) axis (Rivest and Rivier, 1993a). As a result gonadotropin releasing hormone (GnRH) neurons are affected, leading to a reduction in GnRH secretion which is followed by decreased secretion of luteinizing hormone (LH). When the LH surge is suppressed, it results in ovulation failure (Landa and Donoso, 1987). This phenomenon is observed in chronic inflammatory diseases and conditions such as functional hypothalamic amenorrhea (Meczekalski et al., 2008).

Previous studies have shown that IL-1 β most probably acts on the hypothalamus to cause suppression of the LH surge that is critical for ovulation (Kalra et al., 1990; Rivest and Rivier, 1993a; Rivier and Rivest, 1991). Fos expression in GnRH neurons is decreased and GnRH mRNA levels are known to be reduced with IL-1 β treatment (Rivest and Rivier, 1993b). Although IL-1 β could act directly on GnRH neurons to produce this effect, it is equally likely that several neurotransmitters are involved in the cascade of events leading to LH suppression. One such neurotransmitter is norepinephrine (NE). Treatment with IL-1 β decreases NE levels in the medial preoptic area (MPA) of the hypothalamus and this is accompanied by a reduction in LH secretion (MohanKumar and MohanKumar, 2002). However, the mechanism by which this occurs is unclear.

Unlike NE, which is stimulatory to GnRH neurons, GABA is known to be inhibitory to LH secretion (Adler and Crowley, 1986). Therefore it is highly probable that IL-1 β increases GABA levels to suppress the LH surge. However, this possibility has not been investigated before. To study the involvement of GABA in this phenomenon and to clearly understand the interaction between NE and GABA, we used an ovariectomized steroid-primed rat model. We used push-pull perfusion in combination with HPLC-EC to determine the role of both NE and GABA in the IL-induced suppression of the LH surge in conscious, freely-moving animals. To determine the role of GABA receptors, we used specific receptor antagonists to block the effect of IL-1 β .

Results

Location of the push-pull cannula

Fig 1 is a schematic representation of a sagittal section of the rat brain at the level of the MPA indicating the location of the push-pull cannula in animals belonging to different treatment groups. Only those animals that had the push-pull cannula in the MPA were used in the study (80%). Animals that did not have a cannula in the MPA were excluded from the analysis.

Effects of IL-1 β on NE release in the MPA

The effects of IL-1 β on NE levels (pg/min, Mean $\pm$ SE) in the MPA are shown in Fig. 2. The effect of treatment [F (5,184)=18.33], time F (11,108)=15.7] as well as the interaction between treatment and time [F (55,108)=3.07] were found to be statistically significant ($p<0.05$). The pretreatment release levels of NE in the MPA of vehicle and IL-treated rats were not different from each other. After vehicle treatment, NE levels in control animals increased gradually from

1300 h (5.53 ± 0.79), reached a peak at 1630 h (22.49 ± 2.23 ; $p < 0.05$) and declined to 4.8 ± 1.6 at 1800 h. In contrast, treatment with IL-1 β suppressed the rise in NE levels. NE levels in this group were 4.62 ± 1.47 at 1300 h and remained at that level for the remaining period of observation (Fig 2A). The average levels of NE in the IL-treated group (5.33 ± 0.59) was also significantly lower than in the control group (9.514 ± 0.48 ; $p < 0.05$; Fig 2 B).

Effect of IL-1 β on GABA release in the MPA

Changes in GABA levels in the MPA in rats treated with the vehicle or IL-1 β are shown in Fig 3. The effect of treatment [$F(1,47) = 46.71$], time [$F(11,38) = 3.31$] as well as the interaction between treatment and time [$F(11,38) = 3.19$] were found to be statistically significant ($p < 0.05$). The pretreatment release levels of GABA (pg/min, Mean $\pm$ SE) in the MPA of vehicle and IL-treated rats were not different from each other. In the vehicle-treated controls, GABA levels were 62.43 ± 15.87 at 1300 h and decreased to 9.12 ± 16.15 at 1530 h and then returned to pretreatment levels (56.064 ± 12.75) by 1730 h. Treatment with IL-1 β significantly increased GABA levels from 41.82 ± 22.85 at 1300 h to 116.91 ± 13.25 at 1500 h, and by more than 5-fold at 1530 h (235.53 ± 15.5) before declining gradually to basal levels at 1830 h (Fig 3A). GABA levels between 1500-1700 h in IL-1 β -treated rats were significantly higher than that of the vehicle treated control rats ($p < 0.05$, Fig. 3A). Average GABA levels during the entire observation period are presented in fig 3B. In the IL-1 β -treated group, average GABA levels were 189.3 ± 29.14 and these were significantly higher than the levels in the control group (49.88 ± 10.97 ; $p < 0.05$).

Effect of GABA antagonists on IL-1 β -induced changes in NE levels in the MPA

The effects of the two GABA receptor antagonists on NE release in the MPA are shown in Figs 4 A-C. In the group treated with BIC alone, NE levels increased from 4.44 ± 0.93 at 1300 h, by more than three-fold to 18.69 ± 2.6 at 1630 h ($p < 0.05$), and remained elevated (15.01 ± 2.6) at 1700 h. Treatment with IL-1 β +BIC partially reversed the suppressive effect of IL-1 β on NE. NE levels in these animals increased significantly from 4.57 ± 1.21 at 1300 h to 13.31 ± 2.96 at 1630 h ($p < 0.01$). These levels were not different from the group treated with BIC alone at 1630 h but were significantly higher compared to the IL-1 β -treated group (5.41 ± 3.31 ; $p < 0.05$) at this time point (Fig 4A). In the group treated with CGP alone, NE levels increased from 5.2 ± 0.85 to 17.03 ± 2.2 at 1630 h ($p < 0.05$). The levels at 1530 and 1700 h were 13.43 ± 1.21 and 12.06 ± 2.35 , respectively, which were different from the control values at those time points. The combination of IL-1 β +CGP failed to reverse the suppressive effect of IL-1 β on NE release (Fig 4B). The average levels of NE over the entire period of observation are shown in Fig 4C. NE levels in control (9.51 ± 0.48), BIC (9.16 ± 0.83), CGP (9.43 ± 1.14) and in IL-1 β +BIC (6.94 ± 2.2) treated animals were significantly higher than those in animals treated with either IL-1 β alone (5.3 ± 0.59) or IL-1 β +CGP (4.18 ± 0.95 ; $p < 0.05$).

Effect of IL-1 β on plasma LH levels - Role of GABA

Changes in plasma LH levels in animals subjected to the various treatments are shown in Fig 5 A-C. The effects of treatment [$F(5, 163) = 8.3$], time [$F(6, 11) = 11.66$] and the interaction between treatment and time [$F(30, 11) = 3.35$] were found to be significant ($p < 0.05$). In control animals, LH levels (ng/ml, mean $\pm$ SE) were 0.45 ± 0.19 at 1300 h and gradually increased to peak concentrations of 5.9 ± 1.02 at 1800 h ($p < 0.05$), closely following the increase in NE levels in the MPA that occurred at 1630 and 1700 h. In contrast, in IL-1 β treated animals, LH levels were 0.57 ± 0.25 at 1300 h and remained at that level during the rest of the observation period, parallel to the low levels of NE observed in the MPA (Fig 5A).

In the group treated with BIC alone, LH levels increased from 0.99 ± 0.22 at 1300h to 6.82 ± 1.02 at 1700h ($p < 0.05$) and decreased to 4.65 ± 1.18 at 1800h. The levels at 1800h were not different from those in the control animals but the levels at 1700h were higher than those in controls

($p < 0.05$) indicating that the peak in LH was advanced by BIC treatment (Fig 5B). Concurrent administration of BIC in IL-treated rats completely reversed the effects of IL-1. The suppressive effect of IL-1 β on LH was reversed by co-treatment with BIC (Fig 5B). In this group, LH levels at 1800 h (4.68 ± 1.29) were significantly higher than the levels observed in the IL-1 β -treated group at the same time point (0.6 ± 0.7 ; $p < 0.05$). In the group treated with CGP alone, the levels at 1300h were 0.49 ± 0.25 , which increased significantly to 5.65 ± 1.44 at 1800h ($p < 0.05$). Co-administration of CGP with IL-1 did not alter the effects of IL-1 on LH (fig 5C). LH levels in the IL-1 β +CGP-treated rats were similar to the ones treated with IL-1 β alone.

The average levels of serum LH during the entire observation period is shown in Fig 6. Average LH levels in control animals (2.41 ± 0.23), animals treated with BIC alone (2.74 ± 0.39), CGP alone (2.025 ± 0.19) and in animals treated with IL-1 β +BIC (1.64 ± 0.24) were significantly higher than those in animals treated with either IL-1 β alone (0.67 ± 0.05) or IL-1 β +CGP (1.1 ± 0.027 ; $p < 0.05$).

Discussion

The present study explores the mechanisms by which systemic IL-1 β suppresses the steroid-induced LH surge in female rats. It provides evidence for the involvement of GABA, dissects the interaction between NE and GABA and additionally delineates the role of specific GABA receptors involved in transducing the effects of systemic IL-1 β on LH secretion. This is in fact the first study to simultaneously measure NE and GABA levels in the MPA with corresponding measurements of serum LH during a systemic challenge with IL-1 β .

GABA is one of the important regulators of LH secretion. It has been shown to exert an inhibitory tone on the LH surge (Adler and Crowley, 1986; Akema et al., 1990; Demling et al., 1985). Correlation studies in female rats, have shown that GABA levels are low when LH levels increase in ovariectomized steroid primed rats (Demling et al., 1985; Mansky et al., 1982). In addition, GABA release remains low in the MPA during the afternoon of proestrus when LH levels are high (Donoso et al., 1994; Jarry et al., 1988). In contrast, when LH levels are low in diestrus, GABA levels are elevated (Mitsushima et al., 2002). The mRNA for the GABA synthesizing enzyme GAD-67 also decreases in the MPA at the time of the LH surge (Herbison et al., 1992). The inhibitory effect of GABA on LH secretion could be brought about by a direct action on GnRH neurons. In fact, infusion of GABA into the preoptic area can effectively suppress the LH surge in female rats (Herbison and Dyer, 1991). Direct synaptic connections are known to exist between GABAergic nerve terminals and GnRH cell bodies in the MPA (Jennes et al., 1983; Leranthe et al., 1985). Moreover, GnRH neurons in female rats and immortalized GnRH neuronal cell lines express GABA receptors (Favit et al., 1993; Herbison and Dyer, 1991; Jung et al., 1998) indicating the possibility for direct action of GABA on GnRH neurons. The GABA-A receptor agonist, muscimol can suppress the LH surge and also decrease mRNA expression of GABA synthesizing enzymes (Seong et al., 1995). Taken together, these studies indicate that there is a strong likelihood for an inverse relationship between GABA and LH secretion.

There is some indirect evidence to indicate that IL-1 β may act through GABA to suppress the LH surge. A similar immune challenge caused by systemic administration of bacterial lipopolysaccharide increased the synthesis of the GABA-synthetic enzyme, glutamic acid decarboxylase (GAD-67) in the MPA in steroid-primed female rats (Akema et al., 2005; Feleder et al., 1996). More direct evidence comes from studies using whole-cell patch clamp technique, where IL-1 β inhibited the activity of preoptic neurons by increasing presynaptic GABA release (Brambilla et al., 2007; Tabarean et al., 2006). A similar relationship is observed in the present study suggesting that systemic IL-1 β suppresses the LH surge most probably by

increasing GABA levels in the MPA. However, it is not clear if IL-1 β decreases LH secretion by acting on GABA alone. Since LH regulation is highly complex, it is very likely that other neurotransmitters are affected as well.

In this regard, the interaction between GABA and other stimulatory inputs such as NE may be worth exploring because there are several studies that point to the existence of a reciprocal relationship between GABA and NE (Demling et al., 1985; Mansky et al., 1982; Ondo et al., 1982). Also, direct synaptic connections between NE terminals and GABA interneurons have been described (Leranth et al 1988; Horvath et al., 1992). Moreover, administration of the GABA-A agonist, muscimol reduced NE turnover rates, paralleling an attenuation of the LH surge (Adler and Crowley, 1986; Akema et al., 1990). Muscimol administration also blocked the NE-induced LH surge in ovariectomized, steroid-primed rats (Akema and Kimura, 1993). More direct evidence comes from another study in which GABA levels were found to decrease in preoptic perfusates while NE levels increased during the LH surge in ovariectomized, steroid-primed rats (Demling et al., 1985). Also, increasing GABA levels at the level of brainstem noradrenergic neurons, was also capable of suppressing the LH surge (Landa and Donoso, 1987). All these studies taken together support the inverse relationship between NE and GABA that was observed in the present study. A similar relationship could exist in the context of IL-1 β -induced suppression of the LH surge.

To study the relationship between GABA and NE in this phenomenon, we used BIC and CGP which are GABA-A and GABA-B receptor antagonists. While BIC was able to block the effect of IL-1 β on NE levels in the MPA and serum LH, no such effect was observed with CGP treatment, suggesting that IL-1 β 's modulation of the GABAergic system is probably mediated through GABA-A receptors. Several other studies provide substantial evidence for the preponderance of GABA-A receptor over GABA-B receptor in mediating LH secretion. Direct administration of the GABA-A agonist, muscimol, was demonstrated to rapidly reduce LH release and decrease the expression of GnRH in the MPA while the GABA-B agonist, baclofen, did not have such effect (Leonhardt et al., 1995). Moreover, activation of GABA-A receptors is shown to be inhibitory to GnRH electrical activity (Kimura et al., 1993) while GABA-B receptor activation inhibits mRNA expression and peptide synthesis of GnRH (Bergen et al., 1991). Taken together, these studies support an inhibitory role for GABA in LH secretion and the importance of GABA-A receptors in this effect.

Results from the present study indicate that inhibition of the steroid-induced LH surge in female rats by systemic immune challenge is mediated by an increase in GABA levels in the MPA. This increase in GABA most probably causes a reduction in NE release leading to the suppression of LH secretion. This study also provides insight into the receptor mechanisms involved in GABA-mediated inhibition of LH. Specifically, GABA-A rather than GABA-B receptors appear to play a prominent role in mediating the effect of systemic IL-1 β on NE levels in the MPA and serum LH. Thus, systemic IL-1 β is capable of modulating both stimulatory as well as inhibitory inputs on the GnRH system to influence the HPG axis.

Experimental Procedures

Animals—Three-to-four month-old female Sprague Dawley rats, obtained from Harlan Inc. (Indianapolis, IN) were used in these experiments. They were provided food and water ad libitum and were housed in air-conditioned animal rooms maintained at 23 $\pm$ 2°C. The protocols used in this study were approved by the Institutional Animal Care and Use Committee at Michigan State University. After a 2-week acclimatization period, all the animals were randomly divided into six groups.

Stereotaxic Surgery—The animals were bilaterally ovariectomized and implanted with a push-pull cannula in the MPA as described previously (MohanKumar and MohanKumar,

2002; MohanKumar and MohanKumar, 2005). For intracerebroventricular (i.c.v.) injections, the rats were implanted with a stainless steel cannula (22-G) in the lateral ventricle stereotactically (Kopf, Tujunga, CA), using the following coordinates: 1.8 mm posterior, 2.6 mm lateral, 3.6 mm ventral to bregma. After the cannulae were secured with dental cement, a stylet made of 29-G stainless steel tubing was used to plug the guide cannulae to avoid blockage. At the time of the experiment, the i.c.v. stylet was removed and a 29-G inner cannula connected to a Hamilton syringe with polyethylene tubing was used to deliver the drugs used.

Treatment—The steroid treatment protocol was followed as described previously (Kalra et al., 1990; MohanKumar and MohanKumar, 2002). On the 8th day after surgery, the rats were treated with a subcutaneous injection of estradiol-17 β (20 μ g/0.1 ml corn oil) at 1000 h. They were implanted with a catheter in the jugular vein on the 9th day as previously described (MohanKumar and MohanKumar, 2002). On the 10th day, the rats received a subcutaneous injection of progesterone (2 mg/0.1 ml of corn oil) at 1000 h and were subjected to push–pull perfusion. On the day of push–pull perfusion, after collecting a pretreatment blood and perfusate sample at 1300 h, the rats were injected i.p. with either 250 μ l of the vehicle for IL-1 β (PBS-1.0% BSA; control; n=7) or 5 μ g of recombinant rat IL-1 β (Abazyme, Needham, MA; n=6) and i.c.v. with 5 μ L cerebrospinal fluid (CSF). Other groups of rats were treated i.c.v. with either a GABA-A antagonist, bicuculline (Sigma, St Louis, MO) (BIC+saline; 1pg in 5 μ L; n=6), or a GABA-B antagonist, CGP-35348 (Sigma, St. Louis, MO; CGP+saline; 20 μ g in 5 μ L; n=6), in combination with either PBS-1.0% BSA i.p or 5 μ g of IL-1 β i.p (n=5-6 each for IL-1 β +BIC, IL-1 β +CGP) at 1300 h. BIC is a specific GABA-A receptor antagonist (Olsen et al 1978) and CGP35348 binds to GABA-B receptors (Asay and Boyd, 2006). The effective doses of BIC and CGP were titrated based on preliminary pilot studies to ensure that they did not cause any convulsions. The dose of IL-1 β used was based on our previous study (MohanKumar and MohanKumar, 2002).

HPLC: Measurement of NE in push-pull perfusates—NE levels were measured using a Shimadzu HPLC system as described previously (Mohankumar et al., 1994; MohanKumar and MohanKumar, 2004; MohanKumar and MohanKumar, 2005). Briefly, the HPLC set up for the measurement of NE consisted of a phase II, 5 μ m ODS reverse phase C-18 column (Phenomenex, Torrance, CA, USA), a glassy carbon electrode, a CTO-10 AT/VP column oven, a LC-10 AT/VP pump (Shimadzu, Columbia, MD, USA), and a LC-4C amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA). The mobile phase was filtered and degassed through a Milli-Q purification system (Millipore, Bedford, MA, USA) and pumped at a flow rate of 1.8 ml/min. The sensitivity of the detector was 1 nA full scale, and the potential of the working electrode was 0.65 V. The column was maintained at a temperature of 37°C.

Measurement of GABA in push-pull perfusates—The HPLC apparatus for GABA measurement consisted of an ESA model 584 pump (ESA, Chelmsford, MA), a Waters XTerra MS C-18 2.5 μ m 3 $\times$ 50mm column (Waters, Milford, MA), a coulometric electrode model 5014B, microdialysis cell (ESA), and a column oven maintained at 35°C. The data were integrated using a computer with ESA's Coularray for Windows automated software system (ver 2.0, ESA). The mobile phase was made with pyrogen-free water and contained 100mM disodium hydrogen phosphate pH 6.7 with phosphoric acid, 20% methanol, and 3.5% acetonitrile. The mobile phase was then filtered and degassed through a Milli-Q purification system (Millipore Co., Bedford, MA) and pumped at a flow rate of 0.5 mL/min. There was no gain on this system and the potential of the working electrode was 550mV. At the time of analysis, the samples were thawed at room temperature and 19 μ l of perfusate and 1 μ l of the internal standard (Homoserine at 1ng/ μ l; Sigma Chemical Co., St. Louis, MO) were loaded on to the autosampler model 542 (ESA, Chelmsford, MA) with a sample cooler maintaining samples at 4°C. The autosampler performed derivitization of the samples before injection as

follows: addition of 30 µl of working reagent (2.5ml of derivitization reagent and 7.5ml of 0.1M tetraborate buffer) to the perfusates, mixing four times, incubation for one minute and injecting 20µl. The working reagent was made fresh daily. The derivitization reagent consisted of 0.2 M O-phthalaldehyde in 1ml methanol, 0.05% β-mercaptoethanol, and 9ml 0.1M tetraborate buffer pH 9.3. The sensitivity of the system was less than 1 pg/µl.

Histological verification of push-pull cannula implantation site—At the end of the experiment, animals were sacrificed and the brains were removed quickly and frozen immediately using dry ice. Coronal sections of 40 µm thickness were obtained and stained using cresyl violet as described previously (Mohankumar et al., 1994; MohanKumar and MohanKumar, 2002). The stained sections were examined through a light microscope to verify the location of the cannulae with the aid of the rat brain atlas (Paxinos G, 1998).

Statistical analysis—All statistical procedures were performed using SAS software (Cary, NC) unless specified otherwise. Changes in NE, GABA and LH profiles were analyzed by repeated measures ANOVA followed by Fisher's LSD. The average values of NE and LH were compared using one way ANOVA followed by post-hoc Fisher's LSD.

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Fig 1. Push-pull cannula location

Schematic representation of the sagittal section of a rat brain indicating the locations of the push-pull cannulae in control (PBS-1%BSA-treated) group ($\circ$), and animals treated with IL-1 β ($\bullet$), Bicuculline+IL-1 β ($\ominus$) and CGP+ IL-1 β ($\oplus$). Cannula placement in groups treated with Bicuculline and CGP alone are not shown. A1–P3 represent coronal plates extending 1 mm anterior (A1) to 3 mm posterior (P3) to the bregma (AP0). The numbers 8–10 indicate mm ventral to the bregma. MPA=medial preoptic area, Sch=suprachiasmatic nucleus, AH=anterior hypothalamus, LA=lateroanterior hypothalamic nucleus, AVPO=anteroventral preoptic nucleus, MPO=median preoptic nucleus, StHy=striohypothalamic nucleus, VMH=ventromedial hypothalamus, OX=optic chiasm, and SOX=supraoptic decussation. The location of the push-pull cannulae in individual animals was determined by examining stained serial brain sections under a light microscope.

Fig 2. Effects of IL-1 β on NE release in the MPA

Fig 2-A. NE release (mean $\pm$ SE; pg/min) profile in the MPA measured at 30-min intervals in ovariectomized steroid primed animals that were either treated with PBS (Control, n=7), or 5 μ g of IL-1 β (n=6) at 1300 h. 'a' indicates significant difference ($p<0.05$) from levels at 1300 h and 'b' indicates significant difference ($p<0.05$) from levels in control animals. **Fig 2-B.** Average NE levels (mean $\pm$ SE; pg/min) during the entire observation period in control and IL-1 β -treated groups (* $p<0.05$) are shown here.

Fig 3. Effects of IL-1 β on GABA release in the MPA

Fig 3-A. GABA release (mean $\pm$ SE; pg/min) profile in the MPA measured at 30-min intervals in ovariectomized steroid primed animals that were either treated with PBS (Control, n=7), or 5 μ g of IL-1 β (n=6) at 1300 h. 'a' indicates significant difference ($p < 0.05$) from levels at 1300 h and 'b' indicates significant difference ($p < 0.05$) from levels in control animals. **Fig 3-B.** Average GABA levels (mean $\pm$ SE; pg/min) during the entire period of observation in the control and IL-1 β -treated groups (* $p < 0.05$).

Fig 4. Effects of IL-1 β and GABA antagonists on NE release in the MPA

Fig 4-A. NE release (mean $\pm$ SE; pg/min) profile in the MPA measured at 30-min intervals in ovariectomized steroid primed animals that were either treated with bicuculline (veh+BIC, n=6), or a combination of 5 μ g of IL-1 β and bicuculline (IL-1 β +BIC, n=6) at 1300 h. 'a' indicates significant difference ($p<0.05$) from levels at 1300 h and 'b' indicates difference ($p<0.05$) from levels in IL-1 β -treated animals. **Fig 4-B.** NE release profiles in animals that were either treated with CGP-35348 (veh+CGP, n=6), or a combination of 5 μ g of IL-1 β and CGP (IL-1 β +CGP, n=6) at 1300h. Profile in the control group is in the background in grey for comparison purposes. **Fig 4-C.** Average NE levels (mean $\pm$ SE; pg/min) during the entire period of observation in the various treatment groups. * $p<0.05$.

Fig 5. Effects of IL-1 β on serum LH levels

Fig 5-A. Serum LH (mean $\pm$ SE; ng/mL) profile measured at hourly intervals in ovariectomized steroid primed animals that were either treated with PBS (Control, n=7), or 5 μ g of IL-1 β (n=6) at 1300h. 'a' indicates significant difference (p<0.05) from levels at 1300 h and 'b' indicates difference (p<0.05) from levels in IL-1 β -treated animals. **Fig 5-B:** LH profiles in animals that were either treated with bicuculline (veh+BIC, n=6), or a combination of 5 μ g of IL-1 β and bicuculline (IL-1 β +BIC, n=6) at 1300h. The LH profile in control animals is provided for comparison purposes in grey. **Fig 5-C:** LH profiles in animals that were either treated with CGP-35348 (veh+CGP, n=6), or a combination of 5 μ g of IL-1 β and bicuculline (IL-1 β +CGP, n=6) at 1300h.

Fig 6.

Average serum LH levels (mean $\pm$ SE; pg/min) during the entire period of observation in the various treatment groups. * p <0.05.