Interaction among beta-endorphin, nitric oxide and prostaglandins during ovulation in rats

A. G. Faletti, C. Mohn, M. Farina, A. Lomniczi and V. Rettori

Centro de Estudios Farmacológicos y Botánicos (CEFYBO), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Serrano 669, (1414), Buenos Aires, Argentina

The aim of this study was to investigate the relationship between beta-endorphin and nitric oxide (NO) during the ovulatory process in rats. Immature rats were treated with equine chorionic gonadotrophin–hCG to induce ovulation. An intrabursal injection of beta-endorphin stimulated nitric oxide synthase (NOS) activity. This effect was completely reversed when naltrexone was co-injected with beta-endorphin. The stimulatory action of beta-endorphin on NOS activity was studied to determine whether it was exerted via prostaglandins. Treatment with prostaglandin E₂ (PGE₂) completely reversed the beta-endorphin-induced stimulation of NOS activity. Moreover, intrabursal injection of meloxicam, an inhibitor of cyclooxygenase 2, increased NOS activity, but this effect was not altered by co-injection with beta-endorphin. The presence of both endothelial NOS (eNOS) and inducible NOS (iNOS) in the ovary at 10 h after hCG treatment was studied by western blot analysis. Local administration of beta-endorphin inhibited the expression of eNOS protein, whereas expression of iNOS protein was not detectable. Ovarian beta-endorphin content was diminished at 10 h after hCG injection. Treatment with prostaglandin synthesis inhibitors in vivo augmented the ovarian beta-endorphin content. In conclusion, these results indicate that beta-endorphin stimulates the activity of ovarian NO indirectly by inhibiting prostaglandin production.

Introduction

Nitric oxide (NO) is considered to be an important intercellular messenger that controls many physiological processes (Moncada et al., 1991). NO is synthesized through the oxidation of L-arginine by nitric oxide synthase (NOS). NOS occurs in multiple isoforms: neuronal NOS (nNOS) and endothelial NOS (eNOS) are constitutive and calcium–calmodulin dependent (Bredt and Snyder, 1990; Pollock et al., 1991), whereas inducible NOS (iNOS) is calcium–calmodulin independent and cytokine inducible (Stuehr et al., 1991). The rat ovary synthesizes NO and it has been postulated that NO participates in follicular development, ovulation and luteal formation (Ellman et al., 1993; Ben-Shlomo et al., 1994; Shukovski and Tsafiri, 1994; Zackrissen et al., 1996).

Constitutive and inducible NOSs are present in ovarian cells (Van Voorhis et al., 1994) and NOS inhibitors have been shown to reduce the ovulation rate in vivo and in vitro (Shukovski and Tsafiri, 1994; Bonello et al., 1996). Increased concentrations of NO or NO releasing agents can inhibit steroidogenesis in granulosa and luteal cells in both rats and humans (Van Voorhis et al., 1994; Olson et al., 1996). Faletti et al. (1999a) reported that iNOS is the main isoform involved in the ovarian ovulatory process and that the NO produced as a result stimulates the synthesis of prostaglandins that enhance the process of follicle rupture.

Beta-endorphin, a pro-opiomelanocortin (POMC)-derived peptide, has been found in the female reproductive systems of several species. This endogenous opioid is present in sheep (Lim et al., 1983) and human (Aleem et al., 1986) ovaries, in luteal cells of mice (Shaha et al., 1984) and in granulosa and interstitial cells of rodents (Lolait et al., 1985, 1986). mRNA for POMC is also found in the ovary and uterus of mice, monkeys, hamsters (Chen et al., 1986; Jin et al., 1988), rats (Sanders et al., 1990) and fertile women (Galinelli et al., 1995). Previous studies have shown that beta-endorphin may affect ovarian function both directly and indirectly, by modulating LH secretion (Leadem and Kalra, 1985; Gregoraszczuk and Slomczynska, 1998; Kaminski et al., 2000). Beta-endorphin synthesis occurs in ovarian follicles, but the relatively low concentrations found in reproductive tissues indicate that it exerts autocrine or paracrine effects in the ovary (Lim et al., 1983; Lolait et al., 1985; Hamada et al., 1995). Faletti et al. (1995, 1999b) reported that the ovulation rate and the ovarian production of preovulatory prostaglandins were inhibited by beta-endorphin and stimulated by naltrexone. Because NO stimulates the production of prostaglandins, the aim of the present study was to investigate the relationship between beta-endorphin and the NO–NOS system during the ovulatory process in rats.
Materials and Methods

Animals

The animal model used was the same as that described by Faletti et al. (1999a). Briefly, immature female Sprague-Dawley rats were housed in a light (06:00–20:00 h) and temperature (22°C) controlled room, and had free access to laboratory chow and water. At 08:00 h, immature rats of 28–30 days of age were injected i.p. with 15 iu equine chorionic gonadotrophin (eCG) (in 0.15 ml saline) to induce the growth of the first generation of preovulatory follicles. After 48 h, the rats were injected i.p. with 15 iu hCG (in 0.15 ml saline) to induce ovulation, which usually occurs within 12–14 h after hCG administration in this rat colony. The rats were killed by cervical dislocation at 0, 4, 10 or 12 h after hCG injection to measure the ovarian β-endorphin content. Previous studies have shown that gonadotrophin administration increases ovarian NOS activity and prostaglandin content, and that these increases reach a peak at 10 h after hCG administration (Faletti et al., 1995, 1999a). Therefore, this time was chosen as the time before ovulation when NOS activity and prostaglandin content are greatest. Thus, 8 h after hCG treatment (16:00 h), the rats were anesthetized with ether and the compounds to be tested were injected in 50 μl saline into each ovary as described by Shukovski and Tsafirri (1994). Briefly, the rats were anesthetized with ether and each ovary was exposed via a small lumbosacral incision. A 30-gauge needle was introduced into the ovarian bursa via the adjoining fat pad. The local injection was assured by observing the needle tip through the bursa wall and confirmed by the swelling of the bursa. After the injection, the ovary was placed back into the abdominal cavity, and the skin was sutured. After 2 h, these animals were killed by cervical dislocation. The ovaries were removed immediately, frozen on dry ice and stored at −70°C until measurements of NOS activity, western blot analysis or determination of β-endorphin content. The number of animals per group was between five and six per experiment. The experiments were repeated at least twice. All procedures involving animals were carried out according to the NIH guide for the care and use of laboratory animals, and were approved by the Animal Care and Use Committee of Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET).

Drugs and chemicals

Hepes, eCG, hCG, prostaglandin E2 (PGE2), NADPH, N^2^-nitro-L-arginine methyl ester and N^2^-nitro-D-arginine methyl ester (l-NAME and l-NAME), N^G^-methyl-l-arginine and N^G^-methyl-D-arginine (l-NMMA and d-NMMA), L-citrulline, L-valine, indomethacin and di-thiothreitol were purchased from Sigma Chemicals Co. (St Louis, MO). Meloxicam was obtained from Boehringer Ingelheim, and [3H]PGE2 (181 Ci mmol⁻¹) and [14C]arginine (348 mCi mmol⁻¹) from Amersham Pharmacia Biotech (Piscataway, NJ). β-Endorphin for iodination and standards was purchased from Peninsula Laboratories, and Dowex AG50W-X8 resin was obtained from Bio-Rad Laboratories (Hercules, CA). The western blot analysis reagents were obtained from Sigma and Bio-Rad Laboratories.

Nitric oxide activity assay

NOS activity was measured using the conversion of L-[14C]arginine into L-[14C]citrulline assay, according to Bredt and Snyder (1989). On the day when the assay was carried out, both ovaries from each animal were thawed, trimmed of visible fat, weighed and homogenized using a tissue homogenizer with Teflon pestle (Eberbach) in 500 μl buffer Hepes, pH 7.4, containing 0.45 mmol CaCl₂ l⁻¹ and 1.0 mmol dithiothreitol l⁻¹. The homogenates were incubated at 37°C in the presence of [14C]l-arginine (0.2 μCi) and 1 mmol NADPH l⁻¹ in an atmosphere of 5% CO₂ in 95% O₂. Valine (25 mmol l⁻¹), which inhibits the conversion of l-arginine into l-citrulline by arginases, was included in the reaction mixture to increase the specificity of the assay. After 15 min of incubation, samples were centrifuged for 10 min at 7800 g and the supernatant was applied to a 1 ml DOWEX AG50W-X8 column (Na⁺ form) equilibrated with 20 mmol Hepes l⁻¹, pH 7.4, and [14C]citrulline was eluted with 3 ml water. The radioactivity was measured by liquid scintillation counting. Because the formation of l-citrulline is stoichiometric with the formation of NO, it was assumed that an equal amount of NO was formed. The enzyme activity was expressed in pmol NO per g wet mass per min. Intra- and interassay coefficients of variation were < 10%.

Western blot analysis

Soluble tissue extracts were prepared as described by Farina et al. (2001). Briefly, frozen ovaries from each animal were homogenized in 20 mmol ice-cold Tris–HCl buffer l⁻¹, pH 7.4, containing 0.25 mmol sucrose l⁻¹, 1.0 mmol EDTA l⁻¹, 10.0 μg aprotinin ml⁻¹, 10.0 μg leupeptin ml⁻¹, 100.0 μg phenylmethylsulphonyl fluoride ml⁻¹ and 10.0 μg trypsin inhibitors ml⁻¹. The homogenates were sonicated and then centrifuged at 7800 g for 10 min at 4°C. The protein concentrations in the supernatants were determined by the Bradford method using BSA as the standard. Homogenates were heated for 4 min to 100°C in buffer containing 0.3% (w/v) bromophenol blue, 55 μl distilled water and 25 μl β-mercaptoethanol. Equal amounts of protein (100 μg) were loaded on to 4% (w/v) 0.125 mmol Tris–HCl l⁻¹, pH 6.8, stacking polyacrylamide gel, followed by a 7.5% (w/v) 0.375 mmol Tris–HCl l⁻¹, pH 8.8, separating polyacrylamide gel. After
electrophoresis, the proteins were transferred on to nitrocellulose membranes (Pharmacia Biotech, Uppsala) and maintained overnight in a cold chamber using a Bio-Rad transblot apparatus. Membranes were first blocked for 1 h at room temperature in Tris–HCl : saline (50 mmol Tris–HCl 1⁻¹; 500 mmol NaCl 1⁻¹, pH 7.5) containing 5% (w/w) milk powder, and then incubated overnight at 4°C with antibodies raised in rabbit against eNOS and iNOS (Transduction Laboratories, Lexington, KY). The final dilution of both antibodies was 1 : 1000. The membranes were washed three times for 10 min each in Tris–HCl : saline containing 0.1% (v/v) Tween-20, pH 7.5. The membranes were then incubated for 1 h at room temperature with alkaline phosphatase-conjugated anti-rabbit IgG as the secondary antibody and the colour developed with 5-bromo-4-chloro-3-indolyl phosphate toluidine salt and nitroblue tetrazolium. Molecular mass standards were run under the same conditions to identify the protein bands. Blots were scanned using a scanning UMAX Astra 12205 and densitometry was performed using a Dekmate III Sigma Gel software package (Jandel Scientific software). Positive controls for iNOS included homogenates of lipopolysaccharide-activated RAW 264.7 macrophages.

Ovarian content of β-endorphin

The ovaries from each rat treated with eCG–hCG were removed at 0, 4, 10 or 12 h after hCG administration, trimmed of visible fat and the oviduct was weighed and homogenized using a tissue homogenizer with Teflon pestle (Eberbach) in 500 μl cold PBS, pH 7.4, containing a mixture of protease inhibitors (2 mmol EDTA 1⁻¹, 20 mmol N-ethylmaleimide 1⁻¹, 1 mmol phenylsulphonylfluoride 1⁻¹ and 1 mmol dithiothreitol 1⁻¹). The homogenates were heated for 10 min at 100°C and centrifuged for 30 min at 10 000 g at 4°C. The supernatants were purified using Sep-Pak C₁₈ cartridges, lyophilized preparation before being assayed. β-Endorphin was quantified by radioimmunoassay as in previous studies (Faletti et al., 1999b; Lomniczi et al., 2000) using a highly specific antiserum kindly provided by G. Chrousos (National Institute of Child Health and Human Development, Bethesda, MD). The sensitivity of the assay was 20 pg per tube and the curve was linear up to 1000 pg β-endorphin. The antibody crossreacted on an equimolar basis with rat β-endorphin and its immediate precursor, β-lipotropin. Thus, the activity measured in this assay represents the combined concentrations of β-endorphin and β-lipotropin, and is referred to as β-endorphin-like immunoreactivity. Metionine-enkephalin, α-endorphin and other opioid peptides did not crossreact (<0.1% crossreactivity). Human β-endorphin (1–27 fraction) from Peninsula Laboratories (San Carlos, CA) was used for iodination by chloramine T method, because this fraction has two tyrosine residues. Rat β-endorphin has only one tyrosine residue and the iodination of this peptide is very poor. Rat β-endorphin was used for standards and serial dilutions of the samples showed parallelism with the standards curve. The intra- and interassays coefficients of variation were 8.5 and 12.1%, respectively.

Statistical analysis

All data are expressed as means ± SEM. Comparisons between groups were performed using a one-way ANOVA and Student–Newman–Keuls’ multiple comparison test. The difference between the saline group and β-endorphin-treated group in western blot analysis was analysed using Student’s t test. Differences between groups were considered significant when P < 0.05.

Results

Activity of ovarian nitric oxide synthase

Rats were injected intrabursally with saline, β-endorphin (2–200 ng) or β-endorphin (200 ng) plus naltrexone (200 ng) at 16:00 h (8 h after hCG) and ovarian NOS activity was measured 2 h later (10 h after hCG) to determine whether high concentrations of β-endorphin alter the ovarian total NOS activity during the ovulatory process in rats. All doses of β-endorphin significantly increased ovarian NOS activity in comparison with the saline control (Fig. 1). The intrabursal injection of saline solution did not alter NOS activity in comparison with untreated animals. The effect of β-endorphin (200 ng) was completely reversed by co-injection of β-endorphin with naltrexone (200 ng).

Rats were injected intrabursally with meloxicam (0.5 and 5.0 μg), an inhibitor of cyclooxygenase 2, or PGE₂ (0.5 and 5.0 μg), 8 h after hCG treatment to determine whether ovarian prostaglandins modulate the ovarian NO–NOS system, and ovarian NOS activity was measured 2 h later. Both doses of meloxicam induced a significant increase in NOS activity and PGE₂, at the doses used, significantly reduced NOS activity (Fig. 2).

As β-endorphin exerts inhibitory effects on preovulatory prostaglandin production (Faletti et al., 1995, 1997) and increases NOS activity (Fig. 1), it was of interest to determine whether β-endorphin would potentiate the stimulatory effect of meloxicam on NOS activity. The meloxicam-induced stimulation of ovarian NOS activity was not affected by co-injection of meloxicam (5 μg) with β-endorphin (200 ng), but PGE₂ (5 μg) co-injected with β-endorphin (200 ng) reversed the β-endorphin-induced stimulation of NOS activity (Fig. 3).

Immunodetection of nitric oxide synthase proteins

Western blot analysis was used to determine whether changes in eNOS and iNOS correlated with changes in.
was measured by the conversion assay of [14C]arginine into [14C]citrulline in ovarian homogenates and was expressed as pmol of NO per min per g wet mass. Columns represent ovaries treated with saline solution (ss) or βE or βE plus Nal via intrabursal injection. Each bar represents the mean ± SEM for eight to ten animals per group. *P < 0.05, **P < 0.01; aβE versus ss; bβE plus Nal versus βE 200 ng (ANOVA and Student–Newman–Keuls multiple comparison test).

Fig. 2. Effect of intrabursal injection with meloxicam (Mx) (0.5 and 5.0 μg), an inhibitor of cyclooxygenase II, or prostaglandin E2 (PGE2) (0.5 and 5.0 μg) on ovarian nitric oxide synthase (NOS) activity in immature rats treated with equine chorionic gonadotrophin–hCG. The intrabursal injection was administered at 16:00 h (8 h after hCG treatment) and the activity of NOS was measured 2 h later (18:00 h). This activity was measured by the conversion assay of [14C]arginine into [14C]citrulline in ovarian homogenates and was expressed as pmol of NO per min per g wet mass. Columns represent ovaries treated with saline solution (ss), meloxicam (Mx) or PGE2 via intrabursal injection. Each bar represents the mean ± SEM for eight to ten animals per group. *P < 0.05, **P < 0.01; treated versus ss (ANOVA and Student–Newman–Keuls multiple comparison test).

total NOS activity. The antibody to eNOS reacted with the appropriate band corresponding to the 140 kDa protein from the membrane fraction of human endothelial cells (data not shown). A protein band at 140 kDa corresponding to the size of eNOS was expressed at detectable amounts in all ovaries at 10 h after hCG treatment from immature rats primed with eCG and treated with saline or βE (200 ng) (lanes 1 and 2) or β-endorphin (200 ng) (lanes 3–5) 2 h previously (Fig. 4a). The intrabursal injection of saline solution did not alter the eNOS expression in comparison with untreated ovaries (data not shown). Densitometric analysis revealed that the expression of eNOS protein was high in ovaries obtained at 10 h after hCG administration, but that this expression was reduced in ovaries from rats that received an intrabursal injection of β-endorphin (P < 0.05) (Fig. 4b). The antibody to iNOS reacted with the band corresponding to 130 kDa in positive control (homogenates of LPS-activated mouse macrophages), but this expression was not detectable in ovaries obtained at 10 h after hCG administration from immature rats primed with eCG–hCG and treated with saline or β-endorphin (data not shown). When this assay was repeated using a chemiluminescence detection kit (Amersham Pharmacia Biotech) with anti-rabbit IgG horseradish peroxidase-conjugated anti-rabbit
Fig. 4. Effect of intrabursal injection with β-endorphin (βE) on ovarian endothelial nitric oxide synthase (eNOS) protein. (a) Expression of ovarian eNOS protein by western blot analysis at 10 h after hCG from immature rats primed with equine chorionic gonadotrophin–hCG. Rats received an intrabursal injection of either saline solution (ss) (lanes 1 and 2) or 200 ng βE (lanes 3–5) at 8 h after hCG treatment. (b) Quantitative analysis of ovarian eNOS expression after treatment. Data points represent the mean ± SEM; \( n = 5 \) animals per group. * \( P < 0.05 \) (Student’s \( t \) test).

Ovarian content of β-endorphin

β-Endorphin-like immunoreactivity was measured in ovaries of eCG–hCG-treated immature rats to determine ovarian β-endorphin content during the ovulatory process in rats. The ovarian β-endorphin-like immunoreactivity at different times after hCG injection is shown (Fig. 5). At 10 h, the production of β-endorphin-like immunoreactivity was significantly lower than that observed at 0 and 4 h after injection (\( P < 0.001 \)).

Inhibitors of prostaglandins synthesis (indomethacin, meloxicam) and inhibitors of NO production (l-NAME, l-NMMA) were injected intrabursally at 8 h after hCG treatment and the ovarian β-endorphin-like immunoreactivity was measured 2 h later to study whether the reduction in ovarian β-endorphin concentrations during the ovulatory process was related to the increase in prostaglandins and NO production. Ovarian β-endorphin-like immunoreactivity at 0 h in control ovaries and at 10 h after hCG administration in treated ovaries is shown (Fig. 6). Both treatments, indomethacin (10 \( \mu \)g) or meloxicam (10 \( \mu \)g), induced a significant increase in ovarian β-endorphin-like immunoreactivity (\( P < 0.01 \)), but immunoreactivity was lower than that before hCG administration. The hCG-induced inhibition of ovarian β-endorphin-like immunoreactivity was unaffected by treatment with NOS inhibitors (0.3 mg l-NAME or 0.3 mg l-NMMA).

Discussion

NOS activity has been detected in male and female reproductive organs (Burnett et al., 1995; Suburu et al., 1995). In a previous study, it was shown that gonadotrophin administration resulted in an increase in
NOS activity and that this increase reached a peak at 10 h after hCG treatment (Faletti et al., 1999a). The present study investigated the action of β-endorphin on ovarian NOS activity during the ovulatory process. β-Endorphin was injected into each bursa 2 h before hCG treatment, and the results of the present study indicate that β-endorphin stimulates ovarian NOS activity. This effect is mediated largely by μ-receptors, and the major receptor activated by β-endorphin, as naltraxone, a relatively specific μ-receptor antagonist, inhibited the action of β-endorphin on NOS activity. Recent studies indicate that NO is involved directly in the ovulatory process (Shukovsky and Tsafiri, 1994; Bonello et al., 1996; Jablonka-Shariff and Olson, 1998; Drazen et al., 1999; Jablonka-Shariff et al., 1999; Mitsube et al., 1999; Nakamura et al., 1999). Faletti et al. (1999a) reported that NO is involved in ovulation in the rats by stimulating the production of prostaglandins. The ovarian concentration of prostaglandins increases after the LH surge or in response to gonadotrophin stimulation (Bauminger and Lidner, 1975; Brown and Poyser, 1984; Faletti et al., 1995). Recent studies have demonstrated that endogenous and exogenous stimulators of NO production increase the synthesis of prostaglandins by direct activation of cyclooxygenase (Salvemini et al., 1993; Yamouchi et al., 1997). Faletti et al. (1999a) demonstrated that two competitive inhibitors of NOS activity, L-NAME and L-NMMA, inhibited the ovarian production of prostaglandins in vivo and in vitro and that a NO donor, SIN-1 (3-morpholinosydnonimine-hydrochloride), was able to increase the synthesis of prostaglandins in vitro. Other studies using rat uterus also demonstrated the relationship between NO and prostaglandins during implantation, pregnancy and labour (Dong and Yallampalli, 1996; Farina et al., 1998). The mechanisms of action of β-endorphin on NOS activity were investigated to ascertain whether this action was exerted via prostaglandins. Treatment with PGE₂ in vivo completely reversed the β-endorphin-induced stimulation of NOS activity. Moreover, the stimulatory effect of meloxicam or β-endorphin on NOS activity was not additive. Therefore, all of these results indicate that β-endorphin appears to stimulate the activity of ovarian NOS indirectly, by inhibiting prostaglandin production.

The expression of two different isoforms of NOS, iNOS and eNOS, and their regulation by gonadotrophins has been examined extensively in the rat ovary (Van Voorhis et al., 1995; Zackrissone et al., 1996; Jablonka-Shariff and Olson, 1997). Nevertheless, the contribution of each isoform to ovulation is still not clear. Jablonka-Shariff and Olson (1997) used immunohistochemistry and western blot analysis to demonstrate that eNOS and iNOS proteins, but not nNOS, are expressed in the rat ovary and are regulated by gonadotrophins. These workers found that the expression of eNOS increases after eCG-induced follicular development and continues to increase after an ovulatory dose of hCG to reach its maximum expression in the corpus luteum. eNOS is commonly described as a constitutively expressed protein, but the expression of this protein can be altered by various factors. In the present study, the expression of ovarian eNOS was inhibited by local administration of β-endorphin. In previous studies β-endorphin was found to inhibit the uptake of Ca²⁺ in isolated strips of rat uterus (Faletti et al., 1992) and decidual cells (Nandhra and Carson, 2000); therefore, the activity of NOS would be expected to be inhibited. However, injection with β-endorphin into the ovaries of immature rats primed with eCG–hCG resulted in an increase in the total activity of ovarian NOS at 10 h after hCG treatment. On the basis of these results, it was speculated that β-endorphin is acting indirectly on NOS activity via inhibition of prostaglandin production. This activation of NOS could generate excessive amounts of NO which could reduce protein synthesis in a negative feedback mechanism.

Jablonka-Shariff and Olson (1997) have found that during eCG-induced follicular development, iNOS protein content remained relatively constant, but an ovulatory dose of hCG (10 iu) produced an increase in iNOS protein that reached its maximum in the late corpus luteum. In contrast, Zackrison et al. (1996) found that iNOS protein was barely detectable during follicular development and the ovulatory process. Other workers have reported maximal expression of iNOS mRNA in unstimulated ovaries and that this was reduced after hCG injection (Van Voorhis et al., 1995). In the present study, iNOS was not detected in ovaries obtained at 10 h after hCG from immature rats primed with eCG. It is possible that the iNOS protein contents were so low that the sensitivity of the method used was
not sufficient to detect this protein in our biological model.

β-Endorphin-like immunoreactivity in the ovaries of eCG–hCG treated immature rats was measured to study the production of ovarian β-endorphin during ovulation. The β-endorphin-like immunoreactivity diminished at 10 h after hCG treatment and remained at these values until ovulation. Production of ovarian β-endorphin is confirmed by the fact that mRNA for pro-opiomelanocortin was found in the ovaries (Melner et al., 1986; Jin et al., 1988; Sanders et al., 1990). However, it is not possible to eliminate the possibility that the β-endorphin-like immunoreactivity measured may be from other sources. Lovegren et al. (1991) reported changes in ovarian content of β-endorphin at different times after injection with 20 iu eCG and 10 iu hCG 48 h later. If the β-endorphin-like immunoreactivity is expressed as per mg protein or per unit tissue wet mass, a significant decrease was observed after hCG treatment. The presence of β-endorphin in human follicular fluid at concentrations several times higher than those in plasma has been reported (Facchinetti et al., 1986; Kerdelhue et al., 1997). It has been suggested that the ovaries may contribute to the plasma concentrations of β-endorphin during the menstrual cycle of women (Comitini et al., 1989). As β-endorphin exerts an inhibitory action on ovulation, and prostaglandins and NO production are augmented during this process, the present study investigated whether these inflammatory agents diminish opioid peptide content of the ovary. The intrabursal administration of NO inhibitors did not affect the ovarian β-endorphin-like immunoreactivity, but treatment with prostaglandin synthesis inhibitors augmented it. These results indicate that prostaglandins exert a negative downregulation on β-endorphin-like immunoreactivity in the ovary during the ovulatory process. It is important to point out that neither indomethacin nor meloxicam treatment completely reversed β-endorphin-like immunoreactivity to values that were recorded before hCG treatment. Therefore, there must be another pathway independent of the prostaglandin–NO system that reduces ovarian β-endorphin content during ovulation. The administration of naltrexone to immature rats (28–30 days old) advanced the first ovulation in 55–75% of animals (Meijs-Roelofs and Kramer, 1989). These data indicate that endogenous peptides critically restrict LH secretion and may constitute a hypothalamic restraint on the onset of puberty. In addition, previous studies demonstrated that β-endorphin affects ovulation in the rat ovary (Faletti et al., 1995, 1997). All these data indicate that endogenous opioids may be modulating this physiological process, at least in part, by altering ovarian prostaglandin production. The function of ovarian opioid peptides is not known, but they presumably act in an autocrine or paracrine manner in the ovary. The ovulatory process comprises the period between the LH surge (or hCG injection) and follicular rupture. The ovarian NOS activity increases after the LH surge or the gonadotrophin stimulation, and the NO produced stimulates the synthesis of prostaglandins. In turn, the LH surge or hCG administration inhibits the production of β-endorphin, thus avoiding high amounts of this peptide and permitting an efficient induction of ovulation.

The authors thank Daniel E González and J. Fernandez Cueto for their technical assistance. This work has been supported by Grant PEI 0092/97 and BID 802–OC-AR–PICT 00333 from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), PRE-032/99 from Programa Latinoamericano de Capacitación e Investigación en Reproducción Humana (PLACIRH).

References


Bauminger S and Lidner HR (1975) Periovulatory changes in ovarian prostaglandins formation and their hormonal control in the rat Prostaglandins 9 737–751


Bredt DS and Snyder SH (1989) Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum Proceedings National Academy of Sciences USA 86 9030–9033

Bredt DS and Snyder SH (1990) Isolation of nitric oxide synthetase 2 and demonstration that it is constitutively expressed in the testerone-regulated rat prostate Proceedings National Academy of Sciences USA 87 682–685


Chen CL, Chang CC, Krieger DT and Bardin CW (1986) Expression and regulation of proopiomelanocortin-like gene in the ovary and placenta: comparison with the testis Endocrinology 118 2382–2389


granulosa cells. *Journal of Clinical Endocrinology and Metabolism* **63** 1222–1224


Received 18 September 2002.
First decision 20 November 2002.
Revised manuscript received 11 December 2002.
Accepted 20 December 2002.