KOREAN JOURNAL OF ZOOLOGY
Vol. 30, No. 4, 341-350 (1987)

Action of Opiates on LHRH Release *in vitro* and Adenylate Cyclase Activity by Rat Hypothalamus

Kyungjin Kim, Byung Ju Lee and Wan Kyoo Cho
(Department of Zoology, College of Natural Sciences, Seoul National University)

시상하부의 *in vitro* LHRH 분비와 adenylase cyclase 활성에 미치는 opiate의 작용

김 경 진·이 병 주·조 완 규
(서울대학교 자연과학대학 동물학과)

(Received August 20, 1987)

요 약

LHRH 분비와 Adenylate cyclase활성에 미치는 PGE_2, Opioid, 그리고 Ca^{2+}의 영향을 흰쥐의 시상하부 조직을 사용하여 조사하였다. K^+(30mM)에 의한 LHRH 분비촉진은 Ca^{2+} 의존적인데 반하여, PGE_2에 의해 촉진되는 LHRH 분비는 세포의 Ca^{2+}농도에 의존하지 않았다. PGE_2에 의한 LHRH 분비와 cAMP 합성은 PGE_2 농도($1\times10^{-7}M-1\times10^{-4}M$)에 비례하여 촉진되었으며, β-endorphin ($1\times10^{-5}M$)은 PGE_2에 의한 LHRH 분비촉진과 cAMP 합성을 공히 억제하였다. 오피오이드 수용체의 길항제인 Naloxone($1\times10^{-3}M$)은 β-endorphin에 의한 저해효과를 극복시켜서, LHRH 분비와 cAMP 합성은 각각 회복되었으나, cAMP 합성은 부분적인 회복을 보인 반면에, LHRH 분비는 PGE_2에 의한 촉진효과보다도 더 활성화되었다. 결론적으로 LHRH 분비에 미치는 오피오이드의 억제작용은 PGE_2-cAMP의 세포내 전달과정을 저해함으로써 유발되는 것으로 추정된다.

INTRODUCTION

Current evidence suggests that endogenous opioid peptides (EOP) tonically inhibit luteinizing hormone (LH) secretion (Ferin *et al.*, 1984; Meites *et al.*, 1979). This inhibition is mainly based on the observations that administration of opiate antagonists, such as naloxone led to an increase in LH levels whereas exogenous opiates depressed LH secretion

이 논문은 1986년도 문교부 학술연구조성비에 의해서 연구되었음.

(Panerai *et al.*, 1985; Piva *et al.*, 1986). Although the direct effect of EOP on pituitary gonadotrophs cannot be ruled out (Blank *et al.*, 1986; Cacicedo and Franco, 1986), EOP appears initially to act at the level of the hypothalamus. In fact, naloxone promoted luteinizing hormone releasing hormone (LHRH) secretion *in vitro* from the mediobasal hypothalamus (MBH) as well as the median eminence nerve terminals (Cieslak and Pawlikowski, 1986; Drouva *et al.*, 1981; Kalra *et al.*, 1987; Wilkes and Yen, 1981). The cellular action mechanism by which EOP influences the neural LHRH apparatus remains, however, unknown.

A variety of neurotransmitters and several intracellular messengers, such as Ca^{2+}, cAMP and phosphatidylinositol cascade appears to be involved in LHRH secretion (Drummond, 1983; Negro-Vilar *et al.*, 1986; Ojeda *et al.*, 1986; Weiner and Ganong, 1978). For instance, LHRH release from the MBH fragments appears to be a Ca^{2+}-dependent exocytotic process, probably involved Ca^{2+}-influx through voltage-dependent Ca^{2+} channels (Drouva *et al.*, 1984). cAMP generating adenylate cyclase activity is somehow participated in the neural process of LHRH secretion (Ramirez *et al.*, 1985). In fact, the administration of dbcAMP was highly effective in eliciting LHRH release from the superfused MBH derived from ovariectomized estrogen primed rats (Kim and Ramirez, 1985). Adenylate cyclase stimulators, such as forskolin and cholera toxin were also highly potent in stimulating LHRH release from similar preparations (Kim and Ramirez, 1986). Furthermore it has been well documented that the activation of PGE_2 is heavily involved in LHRH release as indicated by *in vivo* as well as *in vitro* studies (Heaubne and Dray, 1984; Kim and Ramirez, 1986; Levine and Ramirez, 1980; Ojeda *et al.*, 1982). Although the molecular mechanism through which prostaglandins are involved in the activation of the LHRH neurosecrtory process remains to be elucidated, it has been postulated that PGE_2 appears to increase intracellular cAMP which in turn triggers LHRH release from the median eminence nerve terminals (Ramirez *et al.*, 1986). EOP inhibits adenylate cyclase activity in the brain tissues as well as neuroblastoma cell lines (Collier and Lay, 1974; Simantov and Levy, 1984; Van Inwegen *et al.*, 1975; Yu *et al.*, 1985). These observations raised the possibility that the inhibitory influence of EOP on LHRH release may be mediated through the cellular pathway of the PGE_2-cAMP generating system.

The present study, therefore, aims: 1) to re-examine the role of Ca^{2+} in the neural process of LHRH secretion evoked by either depolarization or PGE_2 infusion, and 2) to test the hypothesis that EOP would influence the PGE_2-stimulated LHRH release from hypothalamic preparations, presumably through modulating cAMP levels.

MATERIALS AND METHODS

Animals and tissue preparations

Immature female rats of Sprague-Dawley (weight 90~100 g at 24~26 days of age; Seoul

National University Laboratory Animal Center) were maintained in a temperature controlled environment under a 14-h light, 10-h dark photocycle (light on at 06:00 h) with food and water supplied *ad libitum*. Animals at 28 days of age were ovariectomized (OVX) and implanted with Silastic capsules(10 mm in length, id 1.575 mm, od 3.175 mm; Dow Corning) containing 17β-estradiol (235 μg/ml in oil; Sigma) as previously described (Kim and Ramirez, 1982; Ramirez *et al.*, 1980). Two days later, animals were sacrificed at 10:00~10:30 h. Their brains rapidly removed and the mediobasal hypothalamus (MBH) was then dissected as defined previously (Kim and Ramirez, 1982).

Experiments

Exp 1. To examine the functional role of Ca^{2+} in the process of LHRH secretion induced by depolarization, pulses of K^+ (30 mM) were delivered into superfusion chamber containing MBHs (4 units/chamber) continuously perfused with either control or Ca^{2+}-free medium following an one-hr control period.

Exp 2. To further explore the involvement of Ca^{2+} in LHRH release in response to PGE_2, pulses of $PGE_2(1\times10^{-6}M$; Sigma) were delivered into superfusion chamber containing MBHs continuously perifused with either control or Ca^{2+}-free medium. In Exp 1 and Exp 2, an intermittent infusion mode (10 min-on, 40 min-off) was used, and the concentration of PGE_2 ($1\times10^{-6}M$) was a highly effective dose as indicated previously (Kim and Ramirez, 1986).

Exp 3. To examine the hypothesis that EOP may inhibit the PGE_2-induced LHRH release, three experimental groups were employed: 1) PGE_2 (from $1\times10^{-7}M$ to $1\times10^{-4}M$), 2) $PGE_2+\beta$-endorphin ($1\times10^{-5}M$) and 3) $PGE_2+\beta$-endorphin+naloxone ($1\times10^{-3}M$). PGE_2 was initially dissolved in ethanol and aliquots were added to incubation medium to obtain appropriate concentrations. Control tubes contain ethanol at similar concentration. β-endorphin (Sigma) and naloxone (Sigma) were diluted in distilled water and further dissolved with incubation medium to a desired concentration.

Exp 4. To test the hypothesis that an inhibitory influence of EOP on LHRH release may be mediated through adenylate cyclase-cAMP system, hypothalamic P_2 membrane preparations were incubated with either 1) PGE_2 (from $1\times10^{-7}M$ to $1\times10^{-4}M$), 2) $PGE_2+\beta$-endorphin ($1\times10^{-5}M$) or 3) $PGE_2+\beta$-endorphin ($1\times10^{-5}M$)+naloxone ($1\times10^{-3}M$).

Experiments were repeated by either four times (Exp 1 and Exp 2) or more than five times (Exp 3 and Exp 4).

***In vitro* superfusion and static incubation systems**

Details of *in vitro* superfusion has been described previously (Kim and Ramirez, 1985; 1986). Briefly the MBHs were allowed to adjust to the superfusin system for 30~50 min and effluents were then collected on ice at 10 min interval for 4 hr.

In static incubation system, the MBHs were incubated in a polypropylene multiwell plate (Falcon) vials as described previously (Ojeda *et al.*, 1982). The incubation medium was consisted of Krebs-Ringer Phosphate (KRP) (pH 7.4) containing BSA in an atmosphere of

95% CO_2/5% O_2. The tissues were preincubated for 30 min and at the end of this period, the medium replaced by a fresh medium containing the test substrates. The medium were centrifuged at low speed for 10 min and supernatant was assayed for LHRH content. Neither incubation medium nor substances interfered with LHRH radioimmunoassay.

LHRH radioimmunoassay (RIA)

Samples were neutralized to pH 6.5~7.5 with 2N NaOH added to phosphate buffer prior to LHRH RIA. LHRH concentration was determined in duplicate by a RIA procedure (Kim and Ramirez, 1985; 1986) using Chen-Ramirez LHRH antiserum (CRR-11-B-72) at a final dilution of 1:200,000. Synthetic LHRH (Sigma) was used for radioiodination and served as the reference standard. The sensitivity at 80% binding was approximately 0.5 pg/tube. The inter and intra-assay coefficient of variation were 7~8% and 4~5% for 2-pg dose of synthetic LHRH, respectively.

Adenylate cyclase assay and cAMP RIA

Details of this method has been described previously (Kim and Ramirez, 1986). Briefly the MBHs were initially homogenized in ice-cold buffer medium (500 μl) which consists of Tris-HCl (25 nM), n-dithiothreitol(1 mM) and EDTA(1 mM), pH 7.4. The homogenates were centrifuged at 800xg for 10 min at 4°C. The supernatant was centrifuged again at 10,000xg for 10 min at 4°C. The pellets were resuspended with 1 final pellets ml of buffer medium and subjected to another centrifugation at 10,000xg for 10 min. The (designated as P_2 membrane preparation) were reconstituted with 1 ml of buffer and used as source of adenylate cyclase (Daly *et al.*, 1982). Protein content was measured by the method of Bradford (1976) using BSA (fraction V) as the reference standard. Aliquots of P_2 membrane preparation (20 μl) were preincubated with appropriate test agents in incubation medium at 4°C for 30 min. Incubation medium consisted of Tris-HCl buffer (100 mM), $MgCl_2$ (16 mM), EGTA (0.8 mM), BSA (2 ng/ml), n-dithiothreitol (2 mM) and theophylline (20 mM) fortified with GTP (10 μM, pH 7.4). The reaction was initiated by adding ATP (4 mM) into reaction tubes in a shaking water bath at 37°C. After a 15 min incubation, sodium acetate buffer (50 mM, pH 4.75) was added to the assay tubes and the assay tubes were then placed in boiling water bath for 2 min to terminate enzymatic reaction. Supernatant were obtained by centrifugation at 2000xg for 20 min at 4°C and subjected to cAMP RIA. All values were corrected for small amount of endogenous cAMP presents in the control samples which were incubated without ATP or for a cross-reaction of ATP with the antiserum to cAMP.

cAMP concentrations were determined by a validated RIA procedure described previously (Kim and Ramirez, 1986; Steiner *et al.*, 1972). Synthetic cAMP (Sigma) served as the reference standard and were used for radioiodination. The sensitivity of the assay was 0.10 pmol/tube. The inter-and intra-assay coefficients of variation were 5~10% and 4~5% at 5.0 pmol/tube respectively.

Statistical analysis

Student's t-test were used for analysis of data. When more than two groups were considered, an one-way analysis of variance (ANOVA) were employed with a $p<0.05$ required for statistical significance. Fisher's least significant difference test was used for the post-hoc comparison (Kirk, 1969).

RESULTS

An intermittent infusion of K^+ (30 mM; 10 min-on, 40 min-off) resulted in rhythmic release of LHRH from MBH superfused with either control or Ca^{2+}-free medium(Fig. 1). Each pulse of K^+-stimulation significantly ($p<0.01$) promoted *in vitro* LHRH release as compared to its basal level. Pulsatile secretory pattern of both groups was a highly synchronous manner, however the magnitudes of LHRH release from MBH superfused with control medium were significantly higher ($p<0.01$) than those from MBH perfused with Ca^{2+}-free medium. The MBH with PGE_2 resulted in pulsatile release of LHRH, and pulses of LHRH went down markedly compared to the first peak of LHRH. In the condition of either control or Ca^{2+}-free medium, the secretory pattern was indistinguishable indicating that the PGE_2-augmented LHRH release was an extracellular Ca^{2+}-independent manner (Fig. 2).

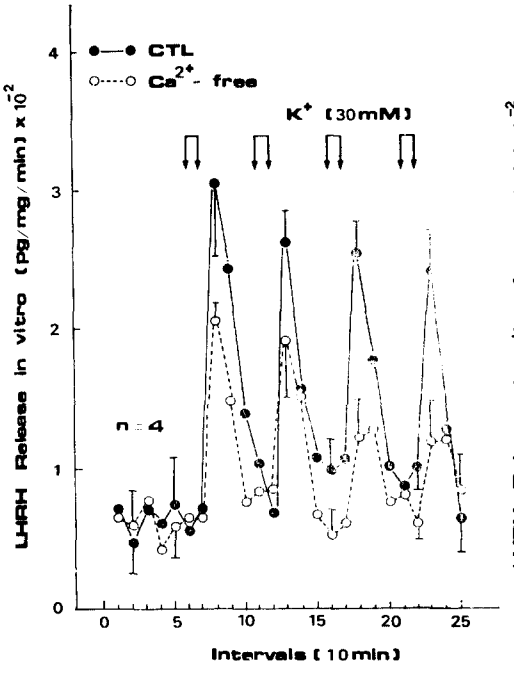

Fig. 1. Effect of K^+ infusion on LHRH release *in vitro* from hypothalamic fragments superfused with either control or Ca^{2+}-free medium.

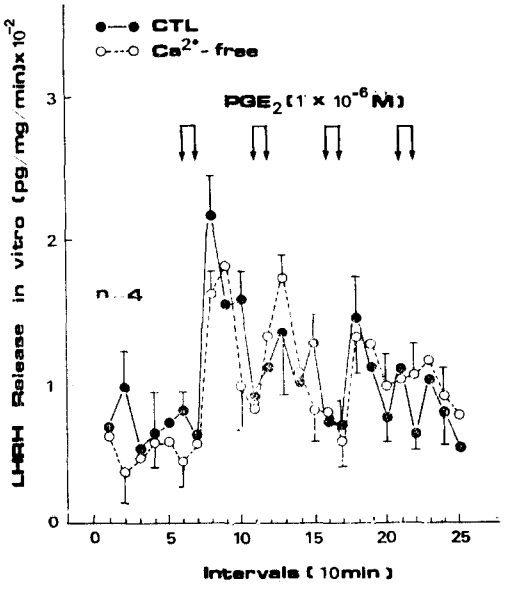

Fig. 2. Effect of PGE_2 infusion on LHRH release *in vitro* from hypothalamic fragments superfused with either control or Ca^{2+}-free medium.

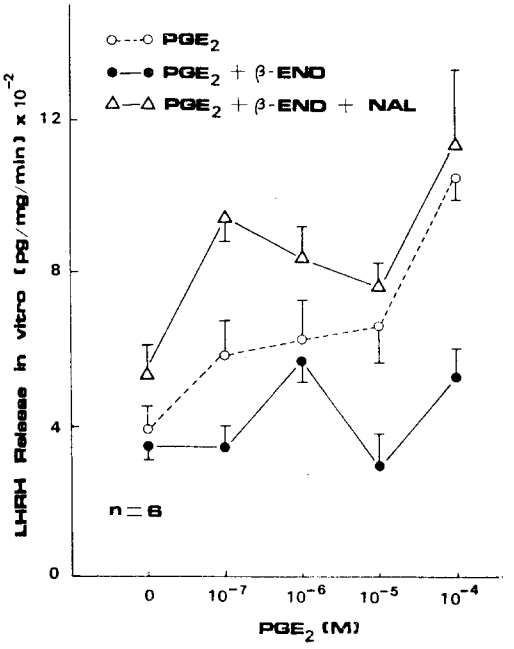

Fig. 3. Effect of β-endorphin and naloxone on the PGE_2-induced LHRH release from hypothalami.

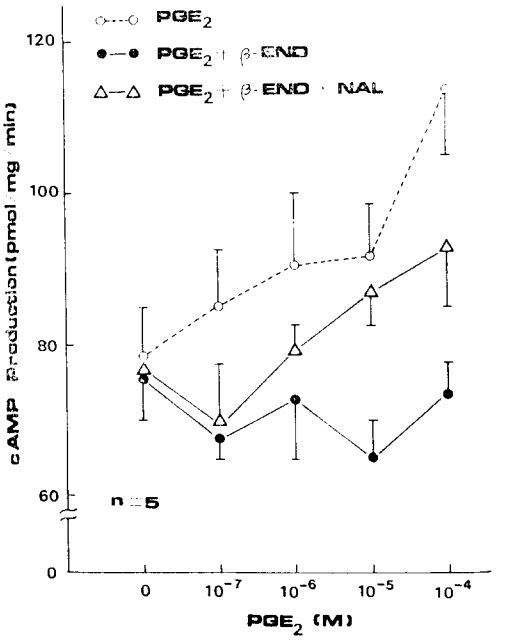

Fig. 4. Effect of β-endorphin and naloxone on the PGE_2-evoked cAMP production by hypothalamic P_2 membrane preparations.

The MBH incubated with increasing doses of PGE_2 (from 1×10^{-7}M to 1×10^{-4}M) promoted LHRH release in a dose-related manner. When β-endorphin (1×10^{-5}M) were added to the medium, PGE_2-enhanced LHRH release from MBH was clearly attenuated except at 1×10^{-6}M of PGE_2(Fig. 3). The basal level remains however unaffected. Addition of naloxone (1×10^{-3}M) counteracted the inhibition of LHRH secretion by β-endorphin. The hypothalamic P_2 membrane preparation with increasing doses of PGE_2 resulted in increment of cAMP production in a dose dependent manner with a clear inhibition by β-endorphin treatment. Administration of naloxone partially antagonized the inhibition of β-endorphin effect on PGE_2-evoked cAMP production, whereas this compound further augmented LHRH release which already increased by PGE_2 (Compare Fig. 3 and 4).

DISCUSSION

The present data demonstrated that EOP plays an important role in presynaptically modulating the neural LHRH apparatus, presumably through PGE_2-adenylate cyclase-cAMP generating system. First, the PGE_2-induced LHRH release was extracellular Ca^{2+}-independent since the reduction in Ca^{2+} influx did not affect the responsiveness of LHRH to PGE_2. However, depolarization induced LHRH release was a clear Ca^{2+}-dependent process, through voltage-dependent Ca^{2+} channels as shown in a variety of experimental models(Rubin, 1972).

Second, EOP such as β-endorphin inhibited LHRH secretion presumably through reduction in adenylate cyclase-cAMP activity. Third, the inhibitory effect of EOP on LHRH release appears to be mediated by specific opiate receptor on the MBH since naloxone, a potent mu-receptor antagonist was highly effective in antagonizing the β-endorphin action on LHRH release as well as cAMP production.

Interestingly, the administration of naloxone further potentiated LHRH release induced by PGE_2, but partially attenuated the inhibition of cAMP production by β-endorphin suggesting that a cellular pathway by adenylate cyclase-cAMP may not be the sole pathway in the neural regulation of LHRH secretion. In fact, it has been recently shown that the phosphatidylinositol cascade is involved in activation of the neural LHRH apparatus (Negro-Vilar *et al.*, 1986; Ojeda *et al.*, 1986). It is, however, of interest to note that activation of protein kinase C by phorbol ester was ineffective in increasing PGE_2 levels, whereas it apparently augmented LHRH release from the median eminence nerve terminals (Ojeda *et al.*, 1986). Therefore it seems possible that two cellular pathways, such as diacyl glycerol-protein kinase C activation and PGE_2-cAMP system may operate in a complementary manner. The interactions between both intracellular transducing system may be the case that LHRH release is maximally enhanced as suggested by Ojeda *et al.*, (1986). Such a complementary pathway is operative as shown in many different experimental models (Michell, 1983; Nishizuka *et al.*, 1984). Although Ca^{2+} appears a key intracellular messengers in the process of neural secretion, the PGE_2-induced LHRH release appears to be not necessarily dependent on extracellular Ca^{2+} influx. Recent evidence provided that mobilization of intracellular Ca^{2+} appears, however, a key step in the PGE_2-induced LHRH release (Ojeda and Negro-Vilar, 1985). Phosphatidylinositol cascade-protein kinase C is also involved in intracellular Ca^{2+} mobilization (Nishizuka *et al.*, 1984). More importantly, it is of interest that β-endorphin induced Ca^{2+} uptake in the brain tissues (Guerrero-Munoz *et al.*, 1979) suggesting that the inhibitory effect of EOP could be exerted through Ca^{2+} mobilization.

It has been well documented that many neurotransmitters participate in the control of LHRH release in particular catecholamines are the major regulatory input to the neural LHRH apparatus (Blank *et al.*, 1983; Kalra and Kalra, 1983; Weiner and Ganong, 1978). Ojeda *et al.*, (1982) showed that LHRH release induced by norepinephrine (NE) was completely inhibited by treatment with prostaglandin synthesis inhibitors, such as indomethacin suggesting that PG activity, particularily, PGE_2 may be an essential mediator for the NE-induced LHRH release (Heaulme and Dray, 1984; Negro-Vilar, 1982). Furthermore the accumulation of cAMP elicited by NE or α-adrenergic receptor agonists was virtually abolished by treatment of hypothalamic slices with indomethacin (Partington *et al.*, 1980). Therefore it is tempting to postulate that PGE_2 may be required an intermediary for the NE-stimulated cAMP production in the hypothalamus. Since the action of EOP on LHRH release may be initially mediated through modulation of NE(Kalra and Simpkins, 1981; Miller *et al.*, 1985), it is feasible to speculate that the activation of opiate

receptor by EOP may inhibit the adenylate cyclase system to decrease cAMP levels which in turn attenuate LHRH release from the median eminence nerve terminals.

ABSTRACT

The present study attempts to elucidate: 1) the possible role of Ca^{2+} on PGE_2-induced LHRH release and 2) the inhibitory influences of endogenous opioid peptides (EOP) on LHRH release as well as cAMP production. Depolarization by K^+ (30 mM) induced LHRH release was Ca^{2+}-dependent, whereas PGE_2 (1×10^{-6}M) evoked LHRH release was a Ca^{2+}-independent process since pulses of PGE_2 produced LHRH pulses from mediobasal hypothalamus superfused with either control or Ca^{2+}-free medium. PGE_2 evoked a dose-related increase in LHRH release and cAMP production. When β-endorphin (1×10^{-5}M) were added to different doses of PGE_2, the increment of LHRH by PGE_2 was abolished as paralleled with inhibition of cAMP production. Naloxone (1×10^{-3}M) antagonized the inhibitory effect of β-endorphin on LHRH release and cAMP production. Interestingly naloxone further potentiated PGE_2-evoked LHRH release, whereas it partially attenuated inhibition of cAMP production by β-endorphin.

These data clearly demonstrated that EOP plays an important role in presynaptically inhibiting the neural LHRH apparatus and its cellular mechanism of action by which β-endorphin inhibits LHRH release appears to be mediated by attenuation of PGE_2-cAMP facilitatory pathway.

REFERENCES

Blank, J.S., J.A. Diez and D.L. Roberts, 1983. Monoaminergic antagonists which block naloxone-induced release of luteinizing hormone selectively to hypothalamic opiate receptors. *Brain Res.* **279**:153-158.

Blank, M.S., A. Fabbri, K.J. Catt and M.L. Dufau, 1986. Inhibition of luteinizing hormone release by morphine and endogeneous opiates in cultured pituitary cells. *Endocrinology* **118**:2097-2101.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* **72**:248-254.

Cacicedo, F. and F.S. Franco, 1986. Direct action of opioid peptides and naloxone on gonadotropin secretion by cultured rat anterior pituitary cells. *Life Sci.* **38**:617-625.

Cicero, T.J., D.P. Owens, P.F. Schmoeker and E.R. Meyer, 1983. Morphine-induced supersensitivity to the effects of naloxone on luteinizing hormone secretion in the male rat. *J. Pharmacol. Exp. Ther.* **225**:35-41.

Cieslak, D. and M. Pawlikowski, 1986. Opioids attenuate whereas naloxone enhances the PGE_2-induced gonadoliberin release from the median eminence area superfused *in vitro*. *Neuroendocrinol. Lett.* **8**:157.

Collier, H.O.J. and A.C. Roy, 1974. Morphine-like drugs inhibits the stimulation of E prostglandins of cyclic AMP formation by rat brain homogenate. *Nature* **248**:24-27.

Daly, J.K., W. Padgett and K.B. Sermon, 1982. Activation of cyclic AMP-generating systems in brain membranes and slices by diterpene forskolin, augmentation of receptor-mediated responses. *J. Neurochem.* **38**:532-544.

Drouva, S.V., J. Epelbaum, E. Laplante and C. Kordon, 1984. Calmodulin involvement on the Ca^{2+}-dependent release of LHRH and SRIF *in vitro*. *Neuroendocrinology* **38**:189-192.

Drouva, S.V., J. Epelbaum, L. Tapia-Arancibia, E. Laplante and C. Kordon, 1981. Opiate receptor modulate LHRH and SRIF release from mediobasal hypothalamic neurons. *Neuroendocrinology* **32**:162-167.

Drummond, G.L., 1983. Cyclic nucleotides in the nervous system. *Adv. Cycl. Nucleot. Prot. Phosphor. Res.* **15**:373-494.

Ferin, M., D. Van Vugt and S. Wardlaw, 1984. The hypothalamic control of the menstrual cycle and the role of endogeneous opioid peptides. *Recent Prog. Horm. Res.* **40**:441-485.

Guerrero-Munoz, F., M. De Lourdes Guerrero, E.L. Way and L.H. Li, 1979. Effect of-endorphin on calcium uptake in the brain. *Science* **206**:89-91.

Heaulme, M. and F. Dray, 1984. Noradrenaline and prostaglandin E_2 stimulate LHRH release from rat median eminence through distinct 1-alpha-adrenergic and PGE_2 receptors. *Neuroendocrinology* **39**:403-407.

Kalra, S.P. and J.W. Simpkins, 1981. Evidence for noradrenergic mediation of opioid effects on luteinizing hormone secretion. *Endocrinology* **109**:776-782.

Kalra, 1983. Neural regulation of luteinizing hormone secretion in the rat. *Endocr. Rev.* **4**:311-351.

Kalra, P.S., W.R. Crowley and S.P. Kalra, 1987. Differential *in vitro* stimulation by naloxone and K^+ of luteinizing hormone releasing hormone and catecholamine release from the hypothalami of intact and castrated rats. *Endocrinology* **120**:178-185.

Kim, K. and V.D. Ramirez, 1982. *In vitro* progesterone stimulates the release of luteinizing hormone releasing hormone (LHRH) from superfused hypothalamic tissues from ovariectomized, estradiol-primed prepubertal rats. *Endocrinology* **111**:750-756.

Kim, K. and V.D. Ramirez, 1985. Dibutyryl cyclic adenosine monophosphate stimulates *in vitro* luteinizing hormone-releasing hormone release only from median eminence derived from ovariectomized, estradiol-primed rats. *Brain Res.* **342**:154-157.

Kim, K. and V.D. Ramirez, 1986. Effect of prostaglandin E_2, forskolin and cholera toxin on cAMP production and *in vitro* LHRH release from the rat hypothalamus. *Brain Res.* **386**:258-265.

Kirk, R.E., 1969. Experimental Design. Procedure for the Behavioral Sciences. Brooks/Cole Publ. Co., Belmont CA. pp. 35-69.

Levine, J.E. and V.D. Ramirez, 1980. *In vivo* release of luteinizing hormone estimated with push-pull cannulae from the mediobasal hypothalamus of ovariectomized, steroid-primed rats. *Endocrinology* **107**:1782-1790.

Meites, J., J.F. Bruni, D. Van Vugt and A.F. Smith, 1979. Relation of endogeneous opioid peptides and morphine to neuroendocrine functions. *Life Sci.* **24**:1325-1336.

Michell, R.H., 1983. Ca^{2+} and protein kinase C: two synergistic cellular signals. *Trends Biochem. Sci.* **8**:263-265.

Miller, M.A., P.K. Clifton and R.A. Steiner, 1985. Noradrenergic and endogeneous opioid pathways in the regulation of luteinizing hormone secretion in the male rat. *Endocrinology* **117**:544-548.

Negro-Vilar, A., D. Conte and M. Valenca, 1986. Transmembrane signals mediating neural peptide secretion: role of protein kinase C activators and arachidonic acid metabolites in luteinizing hormone releasing hormone. *Endocrinology* **119**:2796-2802.

Nishizuka, Y., Y. Takai, A. Kishimoto, U. Kikkawa and K. Kaibuchi, 1984. Phospholipid turnover in nerve action. *Recent Prog. Horm. Res.* **40**:301-345.

Ojeda, S.R. and A. Negro-Vilar, 1985. Release of prostaglandin E_2 from the hypothalamus depends on extracellular Ca^{2+} availability: relation to LHRH release. *Neuroendocrinology* **39**:442-447.

Ojeda, S.R. and A. Negro-Vilar, 1985. Prostaglandin E_2-induced luteinizing hormone releasing hormone release involves mobilization of intracellular Ca^{2+}. *Endocrinology* **116**:1763-1770.

Ojeda, S.R., A. Negro-Vilar and S.M. McCann, 1982. Evidence for involvement of alpha-adrenergic receptors in norepinephrine-induced prostaglandin E_2 and luteinizing hormone releasing hormone release from the median eminence. *Endocrinology* **110**:409-412.

Ojeda, S.R., H.F. Urbanski, K.H. Katz, M.E. Costa and P.M. Conn, 1986. Activation of two different but complementary biochemical pathways stimulates release of hypothalamic luteinizing hormone-releasing hormone. *Proc. Natl. Acad. Sci. USA* **83**:4932-4936.

Panerai, A.E., F. Petraglia, P. Sacerdote and A.R. Genazzani, 1985. Mainly mu-opiate receptors are involved in luteinizing hormone and prolactin secretion. *Endocrinology* **117**:1096-1099.

Partington, C.R., M.W. Edwards and J.W. Daly, 1980. Regulation of cyclic AMP formation in brain tissues by alpha-adrenergic receptors: requisite intermediary of prostaglandins of the E series. *Proc. Natl. Acad. Sci. USA* **77**:3024-3028.

Piva, F., P. Limonta, R. Maggi and L. Martini, 1986. Stimulatory and inhibitory effects of opioids on gonadotropin secretion. *Neuroendocrinology* **42**:504-512.

Ramirez, V.D., D. Dluzen and D. Lin, 1980. Progesterone administration *in vivo* stimulates luteinizing hormone releasing hormone *in vitro*. *Science* **208**:1037-1039.

Ramirez, V.D., K. Kim and D. Dluzen, 1985. Progesterone action on the LHRH and the nigrostriatal dopamine neuronal system: *in vitro* and *in vivo* studies. *Recent Prog. Horm. Res.* **41**:421-472.

Rubin, R.P., 1972. The role of calcium in the release of neurotransmitter substances and hormones. *Pharmac. Rev.* **22**:389-428.

Simantov, R. and R. Levy, 1984. Opiate receptor mechanisms: characterization of selected neuroblastoma-glioma cells with a enhanced response to opiate antagonists. In. E.E. Muller and A.R. Genazzani (eds.). Central and Peripheral Endorphins: Basic and Clinical Aspects. Raven Press, New York, pp. 65-70.

Steiner, A.L., C.W. Parker and D.M. Kipnis, 1972. Radio-immunoassay for cyclic nucleotides. *J. Biol. Chem.* **247**:1106-1113.

Van Inwegen, R.G., S.J. Strade and G.A. Robinson, 1975. Effects of prostaglandins and morphines on brain adenylate cyclase. *Life Sci.* **16**:1875-1876.

Weiner, R.L. and W.F. Ganong, 1978. Role of brain monoamines and histamine in regulation of anterior pituitary secretion. *Physiol. Rev.* **59**:905-976.

Wilkes, M.M. and S.S.C. Yen, 1981. Augmentation by naloxone of efflux of LRF from superfused medial basal hypothalamus. *Life Sci.* **28**:2355-2359.

Yu, V.C., M.L. Richards and W. Sadee, 1985. A human neuroblastoma cell line expresses μ and δ opioid receptor sites. *J. Biol. Chem.* **261**:1065-1070.