J.Sci.Soc.Thailand, 21(1995) 243-252

EVIDENCE FOR MORPHINE-INDUCED PRL RELEASE IN CYNOMOLGUS MONKEYS (*Macaca fascicularis*)

SUCHINDA MALAIVIJITNOND[a] AND PUTTIPONGSE VARAVUDHI[b]

[a] *Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.*

[b] *Emeritus Professor, Primate Research Unit, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.*

(Recieved May 15, 1995)

ABSTRACT

The effect of morphine hydrochloride, u-opiate receptor agonist, at doses of 0, 0.2, 0.4, 0.8 and 1.6 mg/kg on serum PRL levels was investigated in adult female, pubertal and adult male cynomolgus monkeys. Nearly all monkeys showed a prompt rise of serum PRL levels as early as 15 min after subcutaneous morphine injection and reached a maximum value within 30-60 min. Morphine significantly increased serum PRL levels in a dose-dependent manner in which it started from dose 0.2 mg/kg. Except for the highest dose treatment (1.6 mg/kg morphine), serum PRL levels were gradually decreased to the basal levels at 6 1/2 h (390 min). In the same dose, morphine produced a higher and longer increment of PRL levels in female monkeys than pubertal and adult male monkeys, respectively. Indeed, serum PRL levels were never exceeded 1,000 mIU/L at any time points of the study onset in all doses treatment in adult male monkeys. These results have provided evidence supporting an involvement of endogenous opiates in the regulation of PRL release and suggested the possibility of difference in sex and age of lactotroph population in the adenohypophysis of these monkeys.

INTRODUCTION

Hyperprolactinemia is a common clinical disorder and has been generally found in association with amenorrhea, hypogonadism and galactorrhea in females as well as males[1-3]. The elevated serum PRL levels in a patient who has no demonstrable pituitary or central nervous system disease or any other recognized cause is so-called idiopathic hyperprolactinemia[2,3], however, may be resulted from disorder of endogenous opiate system. Since, several lines of evidences now support the hypothesis that endogenous opiates participate in the regulation of PRL secretion under many conditions such as suckling[4,5], stressful stimuli[6] and also physiological condition[7,8]. The PRL stimulating effect of opiates is supposed to mediate by the u-receptor[9-12]. Since β-endorphin which has a high relative affinity of μ-site but low value for δ-site clearly establishes to elevate PRL secretion in rat[9,10], non-human primate[11] and human[12]. While met-enkephalin and leu-enkephalin which show a high relative affinity for δ-site rise PRL levels only in the very high dose in the rat[10].

Therefore, the present study was taken to further clearify the action of u-opiate receptor agonist of morphine in controlling PRL release and the sex differences in response of morphine to stimulate PRL secretion in cynomolgus monkeys (*Macaca fascicularis*).

MATERIALS AND METHODS

Animals

Three adult female (13-16 yr.), 3 pubertal male (7-8 yr.) and 3 adult male cynomolgus monkeys (13-15 yr.), weighing 4.5-6.8 kg were randomly selected from the Primate Breeding Colony, Chulalongkorn University. They were housed individually in each galvanized iron cage. The photoperiod was 0600-1800 h light. Temperature and humidity were slightly fluctuated according to the season. The animals were fed daily in the morning with monkey chow (Pokphan Animal Feed Co., Ltd., Thailand) and supplemented in the afternoon with fresh fruit, vegetable and occasionally chicken boiled eggs.

Drug dosages and treatment schedules

Morphine hydrochloride (M.W. : 321.8, kindly supplied by Drug Dependence Research Center, Institute of Health Research, Chulalongkorn University) was completely dissolved in saline and sterile by filtering through 0.22 μm millipore filter. All animals were injected subcutaneously with saline or morphine solution at doses 0.2, 0.4, 0.8 and 1.6 mg/kg respectively in two-week interval. Blood samples were withdrawn (0800-1500 h) by branchial venepuncture in the squeeze-back cage at 0, 15, 30, 60, 90, 150, 270 and 390 min after the drug injection. Blood serum was separated immediately after the blood clotting at the room temperature by centrifugation at 1,000 xg 20 min and kept frozen at -20°C until assayed for prolactin (PRL) by using homogenous RIA technique.

Radioimmunoassay

In this assay, monkey PRL was measured by RIA technique using the human PRL kit from Diagnostic Products Corporation (DPC), USA with minor modification by reduction the sample and reagents volume. Samples from each subject was run in the same assay to minimize inter-assay variation. The coefficients of intra-assay variations were 9.2, 4.6, 7.2 and inter-assay variations were 9.2, 6.0 and 7.7.

Statistical analysis

Student's paired t-test was used to determine the differences between pairs of means (between saline-injected control and the other doses treatment) in each monkey group. p values of <0.05 were considered significant.

RESULT

Control Values

Mean basal PRL level in female monkeys (77.89 $\pm$ 5.41 mIU/L) was significantly higher than adult (38.43 $\pm$ 5.84 mIU/L) and pubertal male monkeys (22.69 $\pm$ 4.89 mIU/L) ($p<0.05$), respectively. There was no change in serum PRL values after saline treatment in female and pubertal male monkeys, whereas they were increased in some points in adult male monkeys (at 15 and 150 min, $p<0.05$) (figure 1).

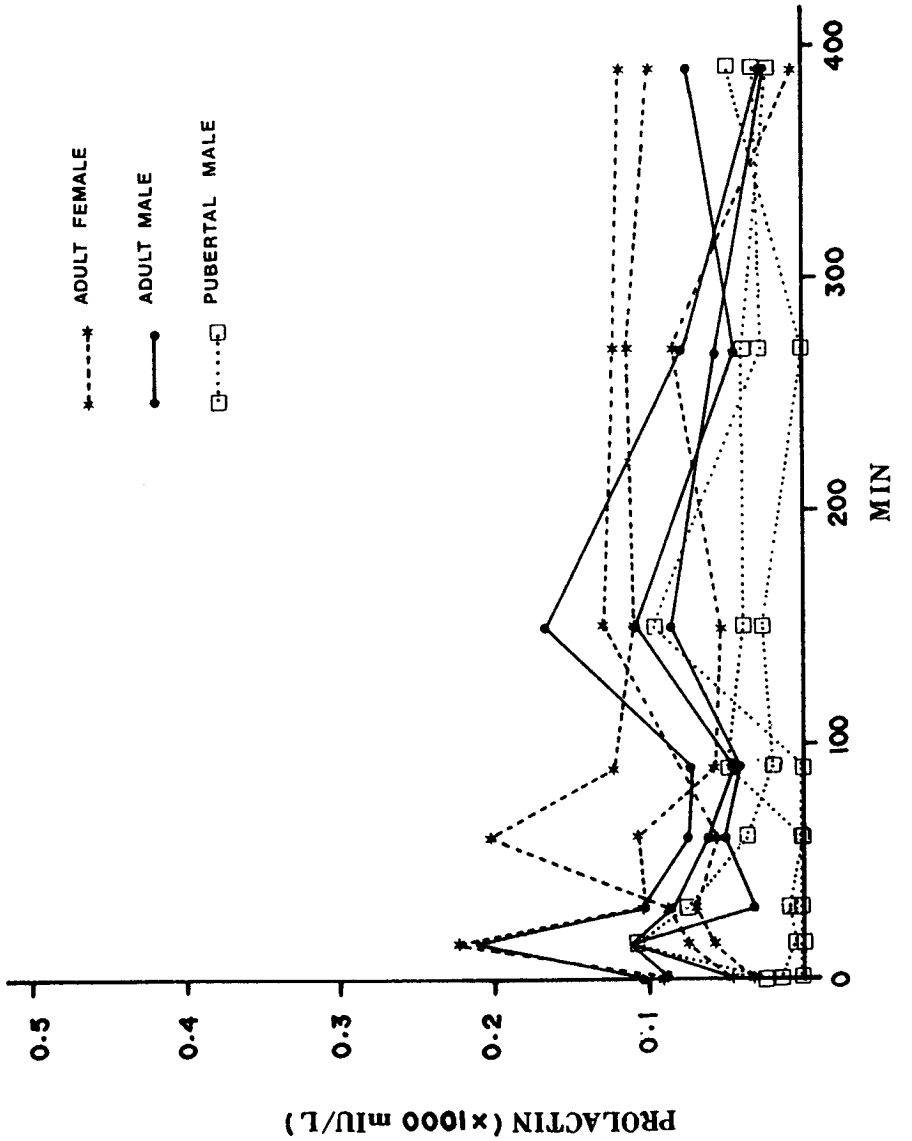

Fig. 1. Serum prolactin levels in adult female, adult male and pubertal male monkeys after subcutaneous injection of saline at time 0.

Effect of Morphine

The effect of single injection of morphine in doses 0.2, 0.4, 0.8 and 1.6 mg/kg on serum PRL levels in adult female, adult male and pubertal male monkeys are shown in figure 2, 3 and 4. In all animals, PRL secretion was stimulated by morphine as low as 0.2 mg/kg but the prominent elevation was observed from dose 0.4 mg/kg on. A prompt rise of serum PRL levels occurred as early as 15 min after subcutaneous morphine injection and reached maximum values within 30-60 min with a subsidence to basal levels thereafter. This increment was in a dose-dependent manner as compared to saline-treated control. Serum PRL values were higher than 1,000 mIU/L after received morphine in dose from 0.4 mg/kg (at 60 min) in female monkeys and dose from 0.8 mg/kg (at 30-60 min) in pubertal male monkeys, respectively. Mean serum PRL levels in adult male monkeys were never exceeded 1,000 mIU/L at any time points of the study onset. In addition, the significant rise of serum PRL levels could be seen as late as 390 min after 1.6 mg/kg morphine injection in female monkeys ($p<0.01$). It was of interest that serum PRL levels showed a sharp peak after morphine treatment in only pubertal male monkeys (figure 4).

In general, galactorrhea symptom was never found after a single morphine injection in all monkeys.

DISCUSSION

These results confirm the previous reports that morphine causes a rapid rise of serum PRL levels[14-16]. In comparison of the pattern of PRL release seen after morphine administration with the pattern seen after buprenorphine administration in the previous report, it may conclude that the effect of morphine is mediated through the μ-opiate receptor. Since morphine has the very high relative μ-affinity of 0.97[17] and the increment of PRL levels in this study wereina dose-dependent manner in all monkeys. Whereas buprenorphine which could interfere with two different, but inter-dependent receptors : at low doses could act at one receptor site (μ-receptor), whereas at higher doses could interact with the second lower affinity receptor (K-receptor), it increased serum PRL levels following the lowest doses and decreased serum PRL levels following the highest doses[18,19]. Additionally, a complete suppression of PRL response to morphine is observed in the rats given of β-funaltrexamine or naloxazone, irreversible, selective and long-acting antagonists of the μ-receptor, 24 hours beforehand. While a preferential δ-receptor antagonist compound, ICI 154,129, has no effect on PRL response to morphine[13].

In agreement with the subsequent data indicating that pituitary lactotroph cells was inhabited in female more than in male[20], the basal PRL values and PRL elevation after morphine injection in female monkeys showed a higher and longer increment than pubertal and adult male monkeys, respectively. However, this effect may also include an estrogen stimulated PRL secretion in female[21,22]. The elevation of PRL levels after morphine injection in pubertal male tended to be higher than adult male. The reason why this difference was existing between ages is still not known, since the basal PRL values did not show any significant difference.

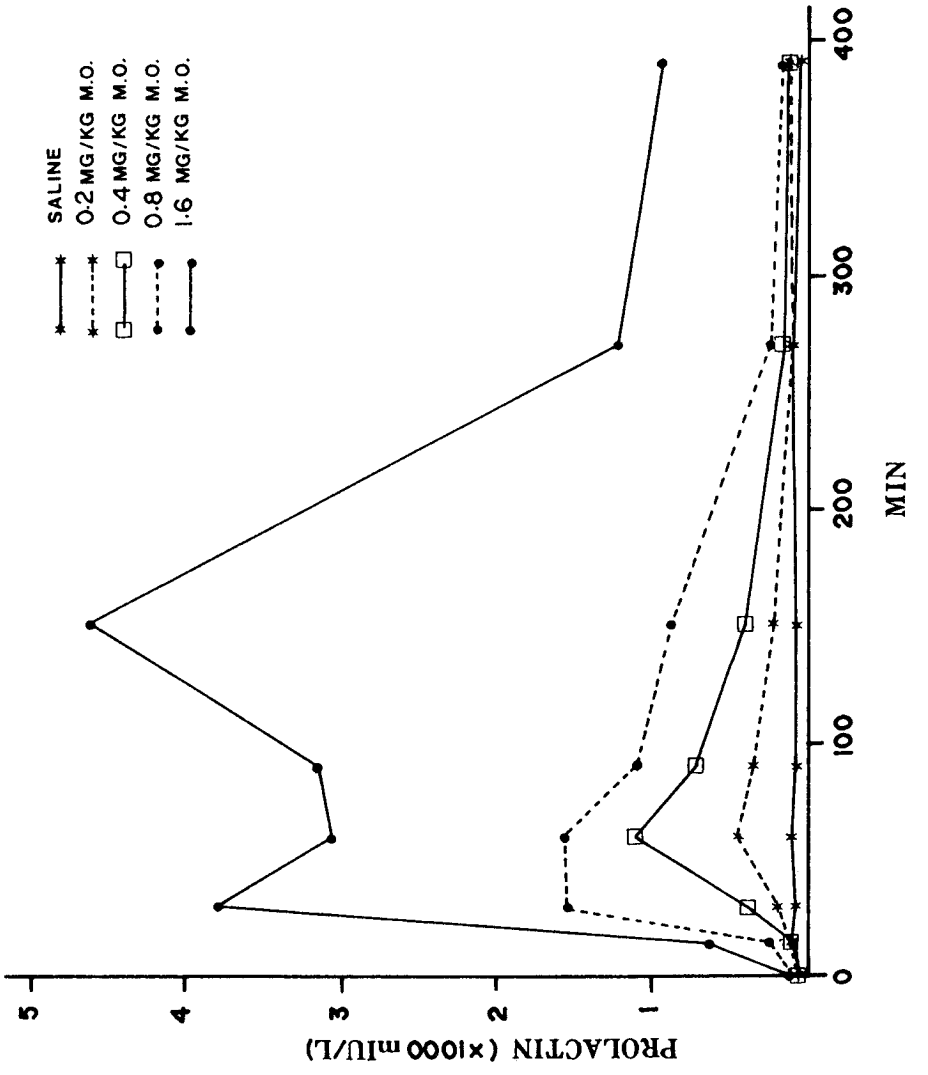

Fig. 2. Mean serum prolactin levels in adult female monkeys after subcutaneous injection of saline or morphine hydrochloride (m.o.) 0.2, 0.4, 0.8 and 1.6 mg/kg, respectively at time 0.

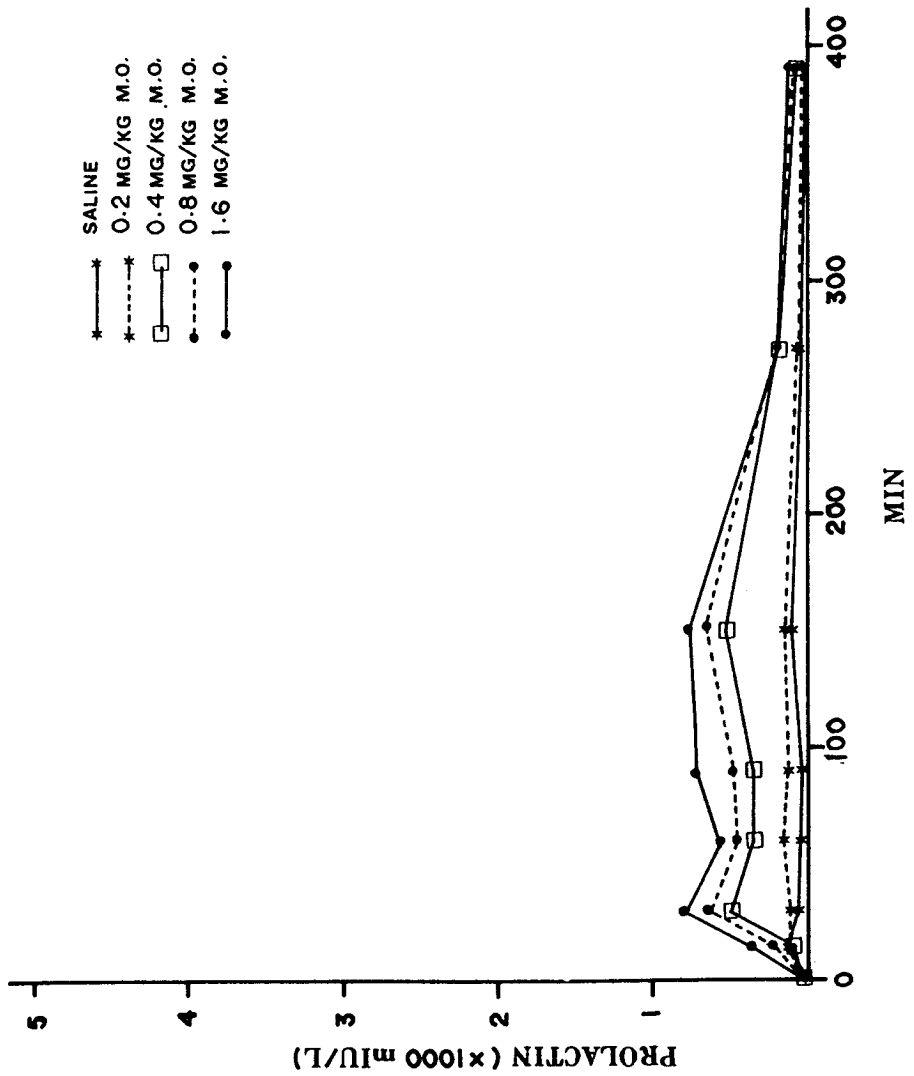

Fig. 3. Mean serum prolactin levels in adult male monkeys after subcutaneous injection of saline or morphine hydrochloride (m.o.) 0.2, 0.4, 0.8 and 1.6 mg/kg, respectively at time 0.

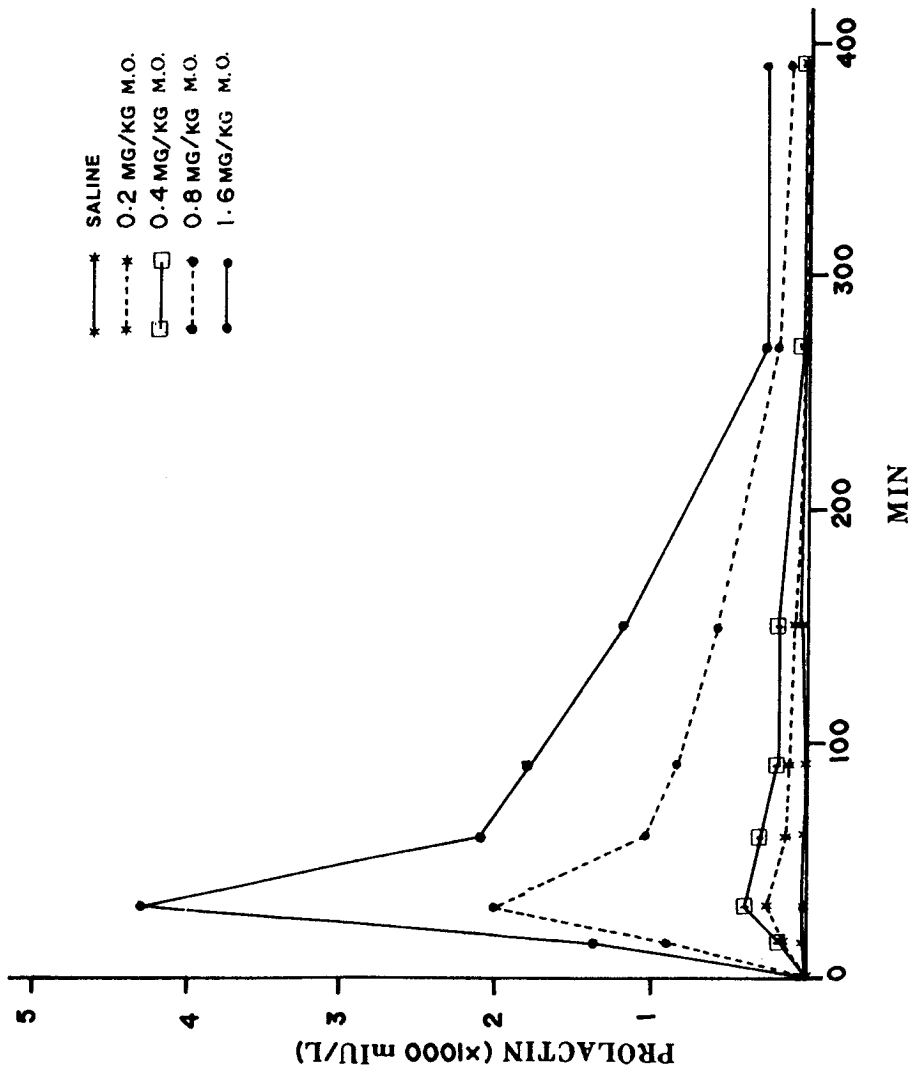

Fig. 4. Mean serum prolactin levels in pubertal male monkeys after subcutaneous injection of saline or morphine hydrochloride (m.o.) 0.2, 0.4, 0.8 and 1.6 mg/kg, respectively at time 0.

Although it could be anticipated that the short-lived prolactin changes have no clinical implication. Our study, however, may provide some insights into the problems of amenorrhea and impotence in addicted persons[23-25]. Since hypogonadism patients has been reported to be often associated with elevated plasma PRL levels[1,2].The action of PRL on testicular steroidogenesis was recently supported by the demonstration of PRL receptors in the interstitial compartment of the testis[26-28]. In addition, we recently found that long-term daily injection of high doses of morphine in both male and female cynomolgus monkeys could show a transient hyperprolactinemia and also induced a galactorrhea symptom[29-31]. Even the dose of morphine treated in those monkeys was very high whencomparedto the dose usage in addicted patients[32], however, the dose-response of PRL secretion in this study could be stimulated by morphine as low as 0.2 mg/kg. Indeed, the other hormonal parameters are harmonized with hyperprolactinemia to induce galactorrhea symptom and the threshold for PRL levels to induce galactorrhea is an individual variation, then, a galactorrhea symptom has been found in only susceptible animals[29-31]. Therefore, the clinical prevalence of galactorrhea symptom related to hyperprolactinemia in narcotic addicted patients should be further elucidated, since it has no reported in human as yet.

REFERENCES

1. Koppelman, M.C.S., Jaffe, M.J., Rieth, K.G. *et al.* (1984). Hyperprolactinemia, amenorrhea, and galactorrhea. *Ann. Int. Med.* **100**, 115-121.
2. Volpe', R., Killinger, P., Bird, C., *et al.* (1972). Idiopathic galactorrhea and mild hypogonadism in a young adult male. *J. Clin. Endocrinol. Metab.* **35**, 684-692.
3. Martin, T.L., Kim, M. and Malarkey, W.B. (1985). The natural history of idiopathic hyperprolactinemia. *J. Clin. Endocrinol. Metab.* **60**, 855-858.
4. Riskind, P.N., Millard, W.J. and Martin, J.B. (1984). Opiate modulation of the anterior pituitary hormone response during suckling in the rat. *Endocrinology.* **114**, 1232-1237.
5. Selmanoff, M. and Gregerson, K.A. (1986). Suckling-induced prolactin release is suppressed by naloxone and stimulated by β-endorphin. *Neuroendocrinology.* 42, 255-259.
6. Van Vugt, D.A., Bruni, J.F. and Meites, J. (1978). Naloxone inhibition of stress-induced increase in prolactin secretion. *Life Sci.* **22**, 85-90.
7. Ragavan, V.V. and Frantz, A.G. (1980). Suppression of serum prolactin by naloxone but not by anti-β-endorphin antiserum in stressed and unstressed rats. *Life Sci.* **28**, 921-929.
8. Guidotti, A. and Grandison, L. (1978). Participation of hypothalamic endorphins in the control of prolactin release. *Adv. Biochem. Psychopharm.* **18**, 191-198.
9. Rivier, C., Vale, W., Ling, N. *et al.* (1977). Stimulation *in vitro* of the secretion of prolactin and growth hormone by β-endorphin. *Endocrinology.* **100**, 238-241.
10. Rauhala,P., Tuominen, R.K.and Mannisto, P.T. (1987). Opioid peptides in the regulation of TSH and prolactin secretion in the rat. *Acta Endocrinologica.* **114**, 383-388.
11. Wardlaw, W.L., Wehrenberg, W.B., Ferin, M. and Frantz, A.G. (1980). Failure of β-endorphin to stimulate prolactin release in the pituitary stalk-sectioned monkey. *Endocrinology.* **107**, 1663-1666.
12. Reid, R.L., Hoff, J.D., Yen, S.S.C., Li, C.H. (1981). Effects of exogenous βh-endorphin on pituitary hormone secretion and its disappearance rate in normal human subjects. *J. Clin. Endocrinol. Metab.* **52**, 1179-1184.
13. Koenig, J.I., Mayfield, M.A., McCann, S.M. *et al.* (1984). Differential role of the opioid mu and delta receptors in the activation of prolactin(PRL) and growth hormone (GH) secretion by morphine in the male rat. *Life Sci.* **34**, 1829-1837.

14. Gold, M.S., Redmond, D.E and Donabedian, R.K. (1979). The effects of opiate agonist and antagonist on serum prolactin in primates : possible role for endorphins in prolactin regulation. *Endocrinology.* **105**, 284-289.

15. Wehrenberg, W.B., McNicol, D., Wardlaw, S.L. *et al.* (1981). Dopaminergic and serotoninergic involvement in opiate-induced prolactin release in monkeys. *Endocrinology.* **109**, 544-547.

16. Tolis, G., Hickey, J. and Guyda, H. (1975). Effects of morphine on serum growth hormone, cortisol, prolactin and thyroid stimulating hormone in man. *J. Clin. Endocrinol. Metab.* **41**, 797-800.

17. Kosterlitz, H.W. (1991). Opioid receptor subtypes : past, present and future. In: Neurobiology of Opioids. Springer-Verlag Berlin Heidelberg, Germany. pp. 123-124.

18. Pechnick, R.N., George, R. and Poland, R.E. (1985). The effects of the acute administration of buprenorphine hydrochloride on the release of anterior pituitary hormones in the rat : evidence for the involvement of multiple opiate receptors. *Life Sci.* **37**, 861-868.

19. Amoroso, S., Renzo,G.D.,Cuocolo, R. etal. (1988). Evidence for a differential interaction of buprenorphine with opiate receptor subtypes controlling prolactin secretion. *Eur. J. Pharm.* **145**, 257-260.

20. Horrobin, D.F. (1973). Prolactin : physiology and clinical significance. The Blackburn Times Press, Great Britain. pp. 7.

21. Sarkar, D.K., Miki, N., Xie, Q. and Meites, J. (1984). Inhibition by estrogen of autofeedback regulation of prolactin secretion. *Life Sci.* **34**, 1819-1823.

22. Thorner, M.O. and Login, I.S. (1981). Prolactin secretion as an index of brain dopaminergic function. In : Neurosecretion and Brain Peptides. Raven Press, New York. pp. 503-520.

23. Cushman, P. (1972). Sexual behaviour in heroin addiction and methadone maintenance. *NYS. J. Med.* **72**, 1261-1265.

24. Martin, W. R. (1973). Methadone-a reevaluation. *Arch. Gen. Psychiatry.* **28**, 268-295.

25. Santen, R.J., Sofsky, J., Bilic, N. and Lippert, R. (1975). Mechanism of action of narcotics in the production of menstrual dysfunction in women. *Fertility and Sterility.* **26**, 538-548.

26. Klemcke, H.G., Amador, A.G. and Bartke, A. (1990). Hormonal regulation of testicular prolactin receptors and testosterone synthesis in golden hamsters. *Bio. Reprod.* **43**, 162-168.

27. Kelly, P.A., Djiane, J., Postel-Vinay, M. and Edery, M. (1991). The prolactin/growth hormone receptor family. *Endocrine Reviews.* **12**, 235-251.

28. Bartke, A. (1980). Role of prolactin in reproduction in male mammals. *Federation Proc.* **39**, 2577-2581.

29. Malaivijitnond, S. and Varavudhi, P. (1993). Sex differences in response of morphine to stimulate prolactin secretion and initiation of galactorrhea in male cynomolgus monkeys. Proc. of the 2nd Intercongress Symposium of the AOSCE, October 26-29. pp.98-99. Chaingmai, Thailand.

30. Setheetham, W. and Varavudhi, P. (1993). Relationship between endogenous PRL secretion, spontaneous galactorrhea and patterns of menstrual bleeding in female cynomolgus monkeys treated with morphine. Proc. of the 2nd Intercongress Symposium of the AOSCE, October 26-29. pp.98-99. Chaingmai, Thailand.

31. Intawat, J. (1994). Long term effects of morphine on serum levels of prolactin, sex steroids and glucose homeostasis in aged female cynomolgus monkeys (*Macaca fascicularis*) and changes after bromocriptine administration. Master's Degree Thesis, Chulalongkorn University (in Thai).

32. Theeravetjaraunchai, S. (1979). A study of circumstances of related drug addiction in patients at Pra Mongkut and Thanyarak Hospitals. Master's Degree Thesis. Mahidol Univeraity (in Thai).

บทคัดย่อ

จากการศึกษาผลของมอร์ฟีนไฮโดรคลอไรด์ซึ่งเป็น μ-opiate receptor agonist ในขนาด 0, 0.2, 0.4, 0.8 และ 1.6 มก./กก. ที่มีต่อระดับฮอร์โมนโปรแลกตินในซีรัมลิงหางยาวเพศเมียวัยเจริญพันธุ์และลิงหางยาวเพศผู้วัยรุ่นและวัยเจริญพันธุ์ตามลำดับ พบว่า ลิงเกือบทุกตัวมีการหลั่งฮอร์โมนโปรแลกตินสูงขึ้นทันทีภายใน 15 นาที หลังจากฉีดมอร์ฟีนเข้าทางใต้ผิวหนัง และมีค่าสูงสุดที่30-60 นาที การเพิ่มระดับสูงขึ้นของฮอร์โมนนี้ขึ้นอยู่กับขนาดของมอร์ฟีนที่ได้รับ หลังจากนั้นจะเริ่มลดลงสู่ระดับปกติที่เวลา 6 1/2 ชั่วโมง (390 นาที) โดยยกเว้นมอร์ฟีนขนาด1.6 มก./กก. ที่ระดับฮอร์โมนยังคงสูงกว่า basal level มอร์ฟีนในขนาดที่เท่ากัน สามารถกระตุ้นให้มีการหลั่งฮอร์โมนโปรแลกตินในลิงเพศเมียได้สูงและนานกว่าลิงวัยรุ่น และวัยเจริญพันธุ์เพศผู้ตามลำดับ และตลอดการทดลองไม่พบค่าของระดับฮอร์โมนโปรแลกตินในลิงหางยาวเพศผู้วัยเจริญพันธุ์สูงเกินกว่า 1,000 mIU/L ผลการทดลองนี้แสดงว่า สารกลุ่มโอปิเอทมีความสำคัญในการควบคุมการหลั่งฮอร์โมนโปรแลกติน และอาจมีความแตกต่างกันระหว่างเพศและวัยในการปรากฏของเซลล์แลกโตโทรปในต่อมใต้สมองของลิงหางยาว