J.Sci.Soc.Thailand, 22 (1996) 285-297

RESEARCH ARTICLES

EFFECT OF KETAMINE ANESTHESIA ON SERUM PROLACTIN LEVELS IN MALE CYNOMOLGUS MONKEYS (*MACACA FASCICULARIS*)

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(Received December 15, 1995)

ABSTRACT

*The effect of ketamine hydrochloride in anesthetic doses (5 and 10 mg/kg) on PRL levels was determined in different ages and under different conditions of male cynomolgus monkeys (*Macaca fascicularis*). Serum PRL levels (cyPRL) were assayed in duplicate using homologous RIA kit for human prolactin (hPRL). The validation of this kit for measuring cyPRL has been done by presenting the parallelism in the immunoreactive of hPRL standard with PRL of cynomolgus monkey from the pituitary extract and from pooled TRH-stimulated serum.*

Serum PRL levels occasionally peaked at 40 minutes after 5 mg/kg as well as 10 mg/kg i.m. ketamine injection and subsided to the basal levels, thereafter. The prominent change was observed only in some monkeys. It did neither depend upon the dosage treatment nor the sexual maturity. However, monkeys having higher basal PRL levels seemingly responded to ketamine in the greater degree than others. It is tempting to speculate that the stimulation of PRL release is depended upon the hormonal pool in pituitary gland in each monkey. Therefore, the data interpretation of this hormone in sequential blood sampling when monkeys are anesthetized with ketamine should be carefully done.

INTRODUCTION

Cynomolgus monkeys (*Macaca fascicularis*) are the particular macaques found in all around Thailand. The characteristic circulating patterns of the principal hormones from these monkeys are virtually indistinguishable from those of human[1]. As a matter of fact, the cynomolgus monkey should be an ideal non-human primate animal model for endocrinic study and transferring the document to human in which the experimental study has been encumbered with moral and ethical standards. However, only the very young monkey and female monkey may be considered gentle and safe, and then, they can be trained to accept the experimental conditions. The male cynomolgus monkey, however, is a very aggressive animal and can hardly be trained to adapt to experimental situations[2]. The procedure preferably utilized to obtain blood sample for hormonal determination in male macaques is a ketamine anesthetization.

Ketamine hydrochloride is an injectable dissociative anesthetic that is widely used in the routine handling of primate[3-5]. It has a high therapeutic index, short-acting and mild properties. Various studies, however, seem to indicate that ketamine may have an effect on hormonal levels in both primate[6-8] as well as non-primate species[9,10]. However, the previous studies in rhesus[5,11-13] and female cynomolgus monkeys[13,14], ketamine was shown an uncertain effect on prolactin (PRL) levels. Ketamine could induce PRL elevation only in some monkeys and no explanation about these results. Accordingly, the present study was designed to clarify the uncertain effect of ketamine in male cynomolgus monkeys on serum PRL levels in different ages and under different conditions.

MATERIALS AND METHODS

Animals

Three pubertal and six adult male cynomolgus monkeys (*Macaca fascicularis*) were randomly selected from the Breeding Colony of the Primate Research Unit, Chulalongkorn University. All of these monkeys were born in the Breeding Colony and used for study the long-term effect of morphine hydrochloride on serum hormonal levels beforehand. These monkeys, therefore, were trained and somewhat habituated to restrain in the squeeze-back cage. In some monkeys, they suddenly offered their limbs through the opening from front part of the cage mesh when the cages were restrained for sampling.

They were house individually in a 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 (purchased from Pokphan Animal Feed, Ltd., Thailand) and supplemented in the afternoon with fresh fruit, vegetable and occasionally boiled chicken eggs.

Experimental design

From the reason, these monkeys were used for studying the long-term effect of morphine hydrochloride on serum hormonal levels by a daily injection of morphine hydrochloride for approximately 100 days beforehand and morphine exerted a particular effect on PRL levels. We, therefore, assumed that lactotroph cells which were mainly the secretory source of PRL hormone of these monkeys were disturbed and changed.

Two months after morphine cessation, these monkeys were administered with 0.5 ml physiological saline. Their blood samples (1.0 ml) were taken frequently at -30, 0, 10, 20, 40, 80, 130 and 180 minutes of saline injection by brachial venipuncture. The monkeys were restrained in the squeeze-back cage for minimal animal handling and environmental changes which caused a stressful effect during the blood collection. One week later, the monkeys were injected with 5 mg/kg ketamine hydrochloride and the blood samples were collected in the same pattern as saline-injection. After that, the monkeys were allowed to recover from the morphine effect for at least 6 months. Then, the monkeys were injected with 10 mg/kg ketamine and the blood samples were taken in the same pattern as described above.

To clarify the effect of stress and ketamine on PRL levels, one monkey (5 year-old) in the colony which was postulated to expose to stress was selected to study the alteration of PRL level after saline and ketamine injection in the same protocol as the previous monkeys. He exhibited an excessive hair loss around his trunk, legs, arms and head. The consecutive blood sampling before this study for 148 days showed the progressive decline in estradiol and testosterone levels, and agreed with the progressive rise in PRL and cortisol levels (figure 1).

Less positive correlation was seen between the levels of cortisol and PRL ($r=0.241$; $p<0.05$) and higher positive correlation was seen between estradiol and testosterone levels ($r=0.545$; $p<0.01$).

The blood serum was separated immediately after the blood clotting at the room temperature by centrifugation at 1,000 xg 20 minutes and stored at -40°C until hormonal assay by RIA technique.

Hormone assay

Serum PRL levels were assayed in duplicate using homologous RIA kit for human PRL developed by Diagnostic Product Corporation, USA. The validation of this kit for measuring serum PRL level in cynomolgus monkeys has been done by presenting the parallelism in the immunoreactivities of human PRL standard with PRL of cynomolgus monkey from the pituitary extract (kindly supplied by Dr.Takashi Yoshida, Tsukuba Primate Center for Medical Science, Japan) and from pooled TRH-stimulated serum. The monkey PRL serum was therefore expressed in the term of Second International Standard for PRL no.83/562 (2ndIS 83/562). The sensitivity of the assay was 14.38 mIU/L. In this study, the inter-assay coefficient of variations were 9.2, 6.0 and 7.7, and intra- assay coefficient of variations were 9.2, 4.6 and 7.2 for the high, medium and low values in the assay, respectively.

All samples in the individual subject were run in the same assay to minimize the inter-assay variation.

Thyrotropin-releasing hormone (TRH) treatment

Eight adult female cynomolgus monkeys were considered to be a subject for preparing high PRL level of quality control. From the reason that the baseline PRL level was higher in females than in males and the peak level response to TRH administration in females was also higher than in males.[15] These monkeys were administered a 100 μg bolus intravenous injection of TRH in 1.0 ml saline. Their blood samples were collected frequently at 0, 10, 20, 30, 60, 90 and 180 minutes, thereafter.

Statistical analysis

Relative potency and their 95% confidence limits were calculated by Bliss method (1952).[16] The statistical analysis for linearity and parallelism was performed according to Sakuma (1964).[17]

RESULTS

Thyrotropin-releasing hormone treatment

A striking and consistent elevation of PRL levels was seen 10 minutes after intravenous TRH injection. The peak values occurred at 20 minutes and thereafter serum PRL levels fell slowly toward baseline by 180 minutes (figure 2). After that, separated blood sera from each monkey during PRL rise were pooled together and using for immunoreactivity check for hPRL kit.

Figure 3 demonstrated a cross-reactivity of PRL of cynomolgus monkey from pituitary extract and from pooled TRH-stimulated serum with antibody to human PRL. The immunoreactivity curve for human PRL was statistically parallel ($p<0.001$) with those of cynomolgus monkey PRL in both from pituitary extract and from pooled TRH-stimulated serum. The index of precision (lambda) in this assay system ranged from 0.07 to 0.10.

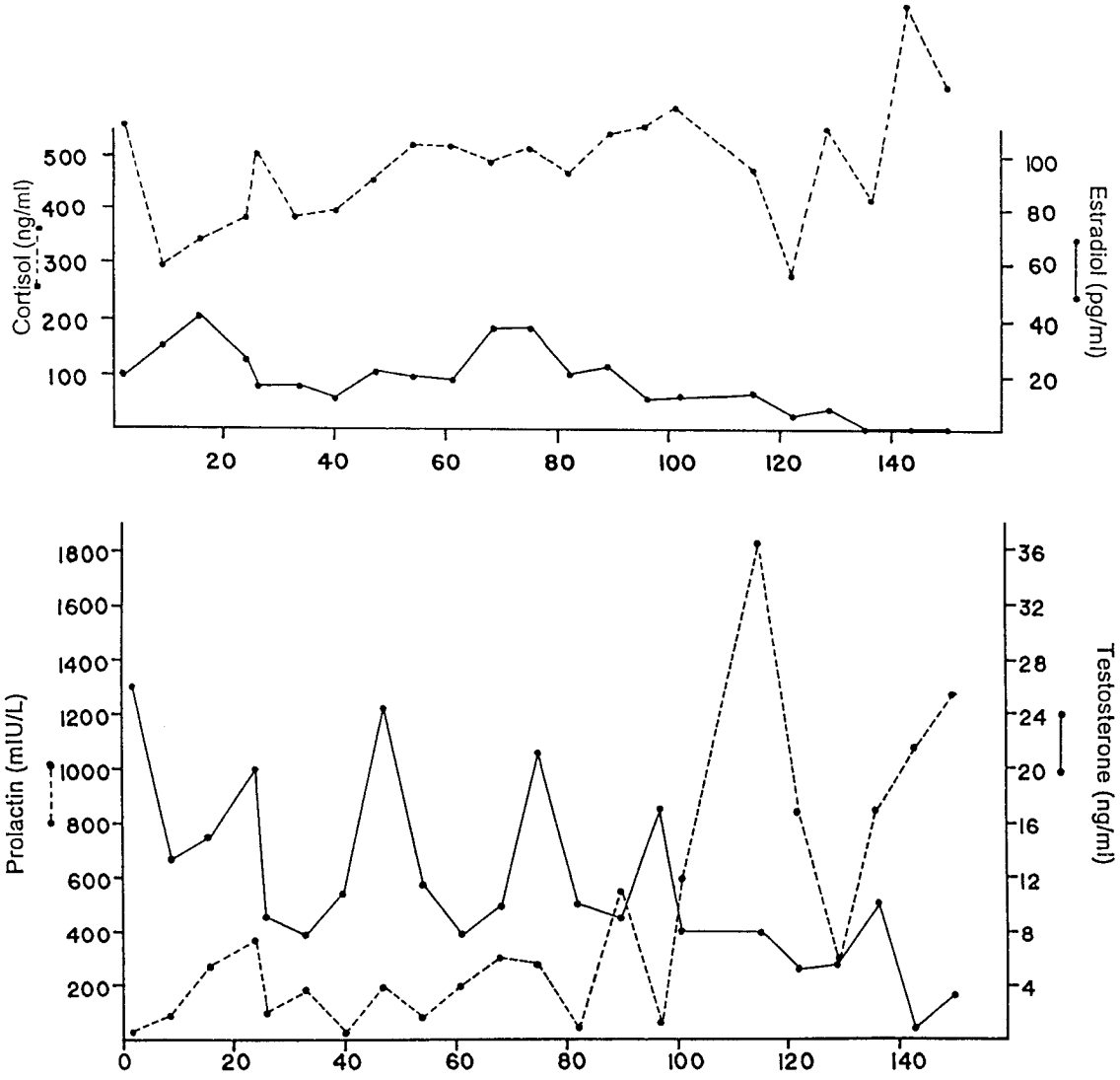

Fig. 1. Serum levels of cortisol, estradiol, prolactin and testosterone in male cynomolgus monkey which was postulated to expose to stress.

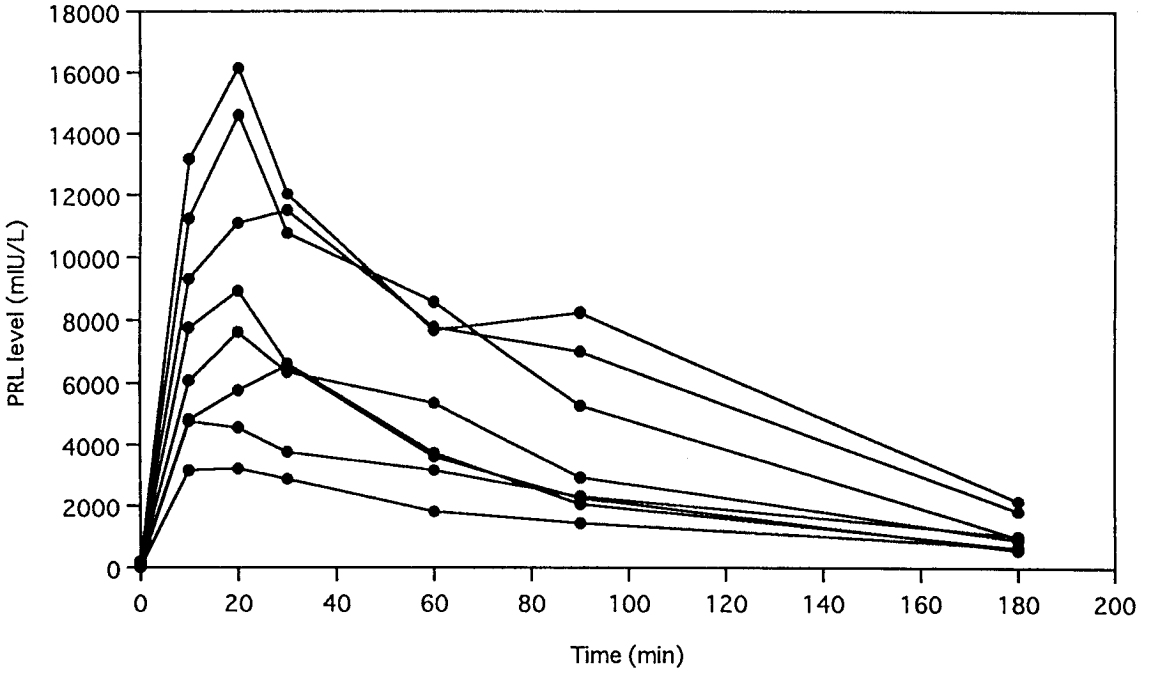

Fig. 2. Serum prolactin levels in adult female cynomolgus monkeys after an intravenous injection of 100 μg TRH at 0 minutes.

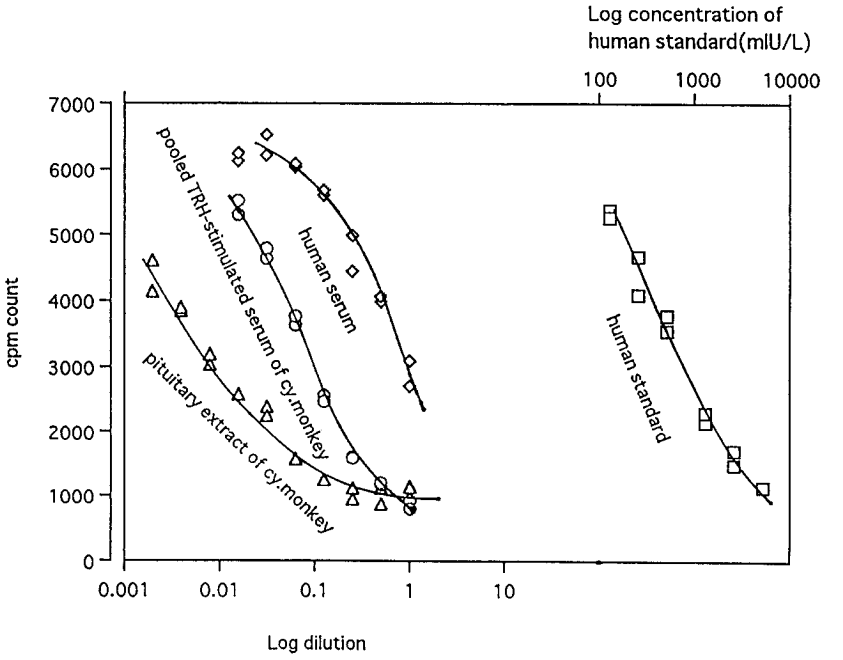

Fig. 3. Comparison of the prolactin immunoreactivity curves of cynomolgus monkey from the pituitary extract and from the pooled TRH-stimulated serum with purified human standard.

Saline injection

There were no discernible changes in PRL levels after saline injection in both adult and pubertal male monkeys (figure 4 and 5). It is of interest to note that the PRL level in adult monkey no.509 was consistently higher than the other monkeys.

Ketamine injection

Serum PRL levels occasionally peaked at 40 minutes after an intramuscular administration of 5 mg/kg as well as 10 mg/kg ketamine hydrochloride (figure 6-9). After that, the PRL level declined progressively to basal control levels at 180 minutes. However, the prominent change was observed only in some monkey and did not depend upon the dosage of ketamine treatment. Some monkey showed an increment of PRL level after 5 mg/kg ketamine injection and did not exhibit any alteration after 10 mg/kg ketamine, or vice versa in the other monkeys. The sexual maturity did not influence the response of PRL levels to ketamine. The pubertal males could response to ketamine resemble to adult males.

The rise in PRL levels was seemingly steeper in the animals having higher initial values (figure 10). For example, in monkey no.509 the basal PRL level was 473.41±36.51 mIU/L (mean±SD) and reached a peak at 1187.52 mIU/L after 5 mg/kg ketamine injection, on the other hand, when the monkey exhibited the basal PRL level as 118.05±40.05 mIU/L the maximal PRL level was only 353.70 mIU/L after the higher dose of ketamine injection (10 mg/kg).

Stress effect

Basal PRL levels in this monkey were high in all various treatment periods (Figure 11). PRL rise was also observed in saline injection with the same levels as 5 mg/kg ketamine injection. But, in saline-treatment condition the PRL level started to increase at 0 minutes, whereas in 5 mg/kg ketamine-treatment condition the PRL level began to increase after the drug treatment (at 10 minutes). Of interest, the PRL level was progressively increased as long as 180 minutes after 10 mg/kg ketamine injection.

DISCUSSION

We have already demonstrated that a commercially available radioimmunoassay kit for human PRL is suitable for measurement of cynomolgus monkey PRL by presenting the parallelism of immunoreactivity curves. Moreover, this radioimmunoassay system could also detect the increment of PRL levels after TRH injection in female cynomolgus monkey. This treatment was preferably used to check the validation of the assay system of PRL hormone.[6,8] We, therefore, applied this kit for determining changes of PRL level after ketamine injection. Walker *et al.* (1987)[8] also used this kit for determining PRL levels in a female baboon. It may suggest that this assay may be a useful assay for PRL hormone from the other primate species as well.

Two months after morphine cessation, all these monkeys were injected with saline and separated blood sera were measured for PRL levels. These PRL levels may hopefully reflect a condition of lactotroph cells in pituitary gland. Dramatically, serum PRL level in monkey no.509 was very high when compared with the other adult and pubertal monkeys. It was only one monkey which continually exhibited milky-excretion from the mammary gland during a daily morphine injection.[18] This evidence furnishes favoring the stimulatory effect of morphine on the activity of lactotroph cells which are mainly the secretory source of PRL. Hence, 5 mg/kg ketamine injection to this monkey in the following week particularly stimulated PRL secretion.

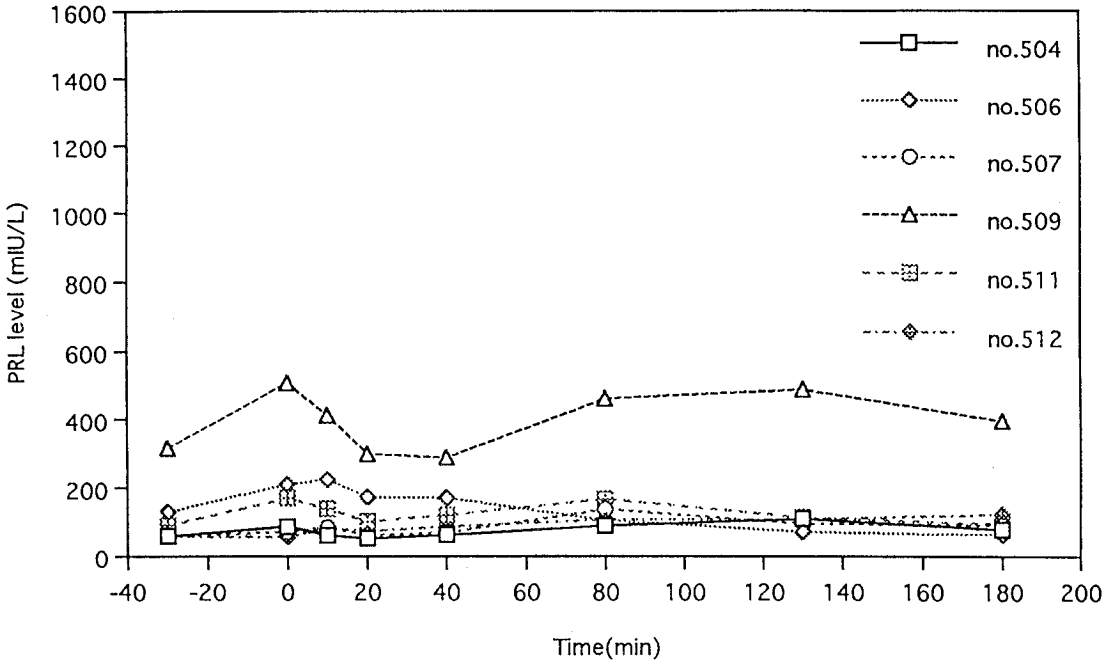

Fig. 4. Serum prolactin levels in adult male cynomolgus monkeys after intramuscular injection of saline at 0 minutes.

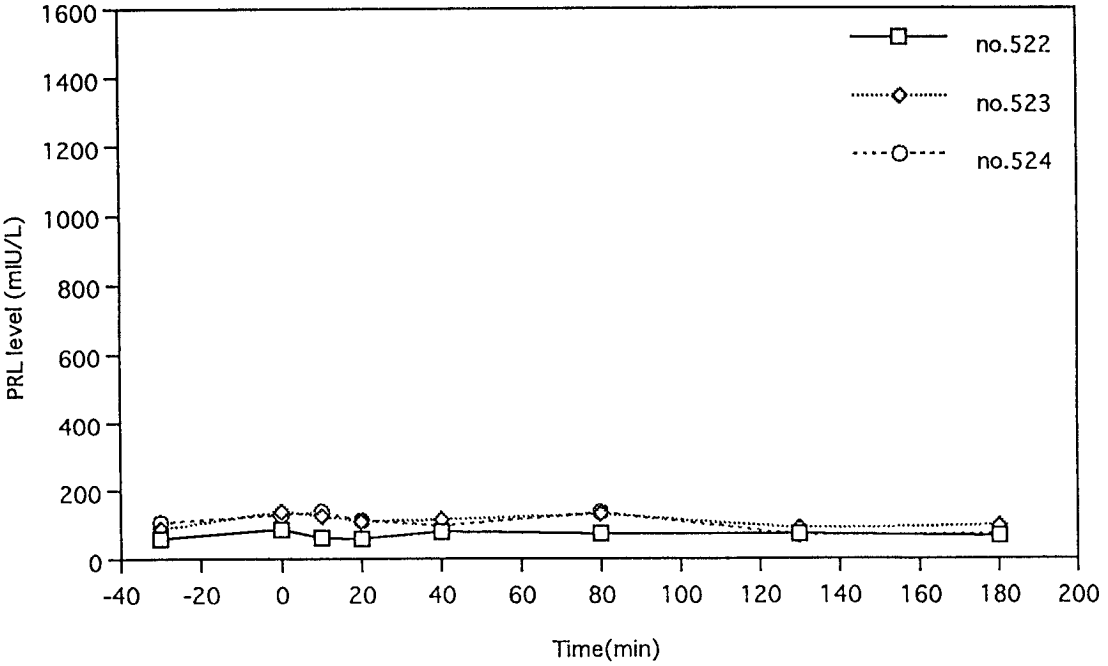

Fig. 5. Serum prolactin levels in pubertal male cynomolgus monkeys after intramuscular injection of saline at 0 minutes.

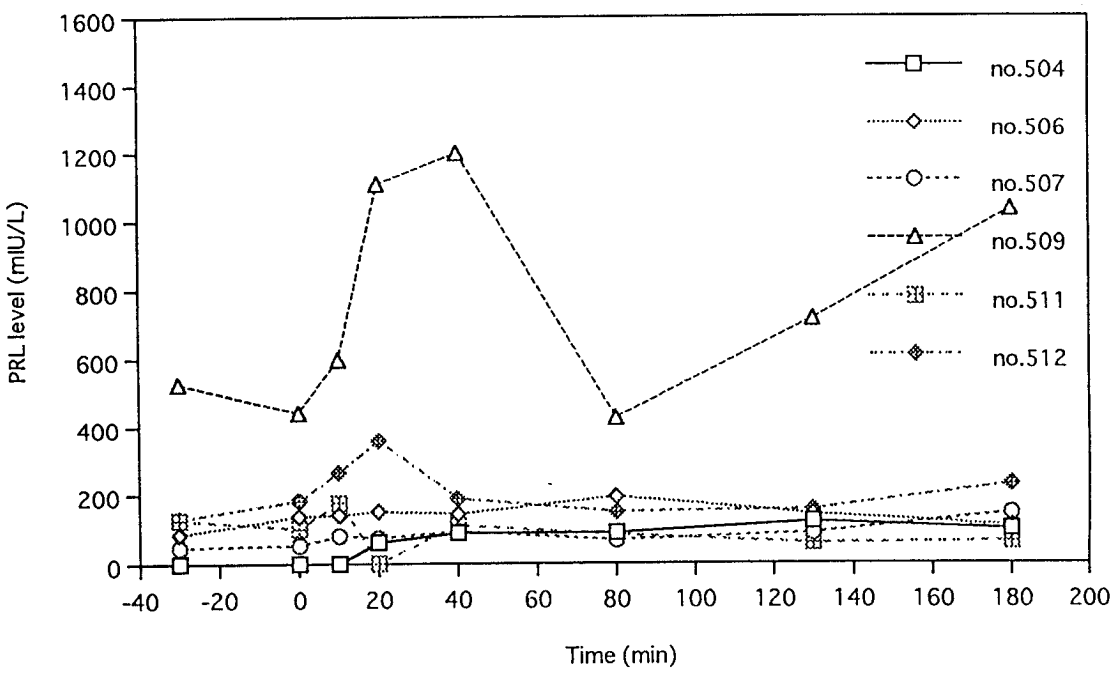

Fig. 6. Serum prolactin levels in adult male cynomolgus monkeys after intramuscular injection of 5 mg/kg ketamine at 0 minutes.

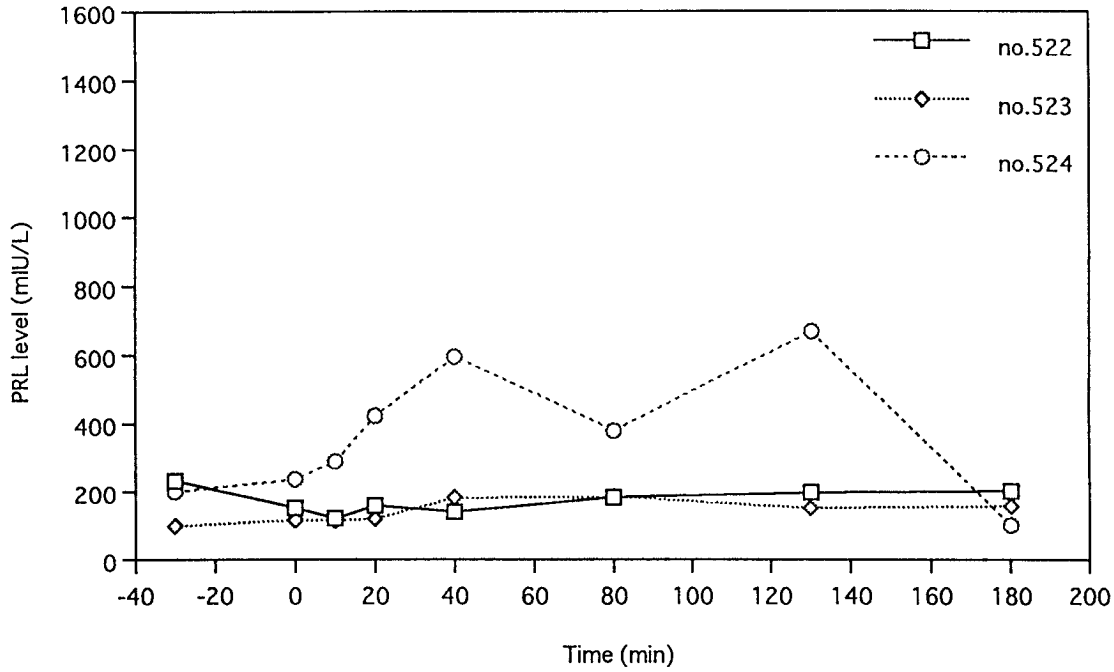

Fig. 7. Serum prolactin levels in pubertal male cynomolgus monkeys after intramuscular injection of 5 mg/kg ketamine at 0 minutes.

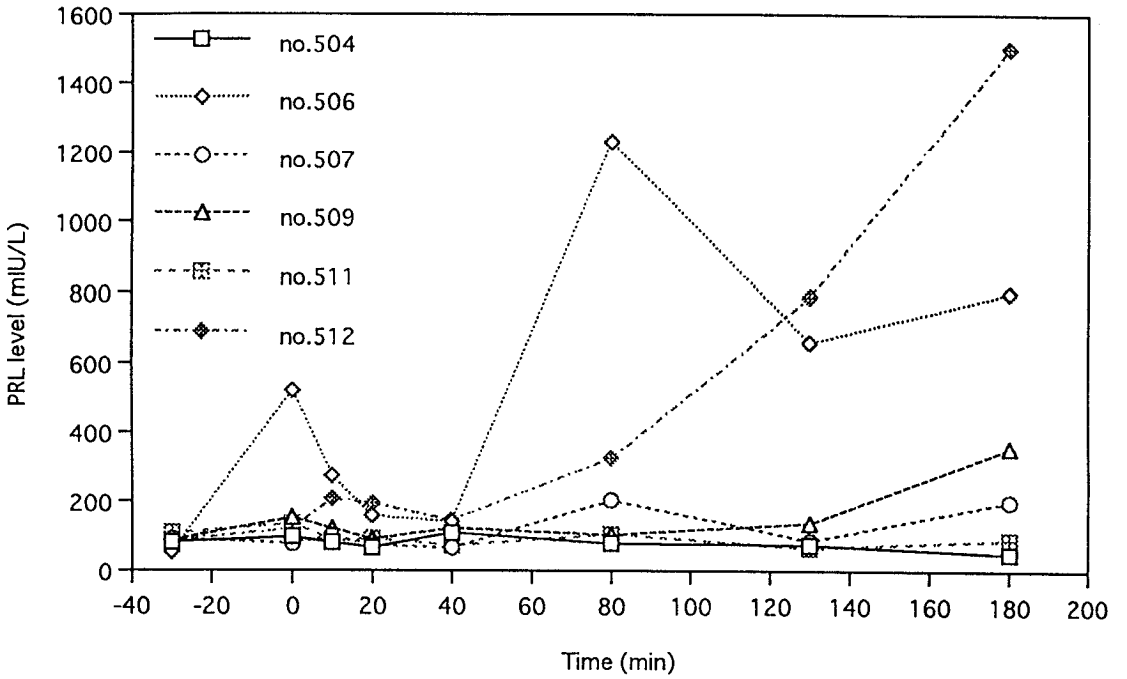

Fig. 8. Serum prolactin levels in adult male cynomolgus monkeys after intramuscular injection of 10 mg/kg ketamine at 0 minutes.

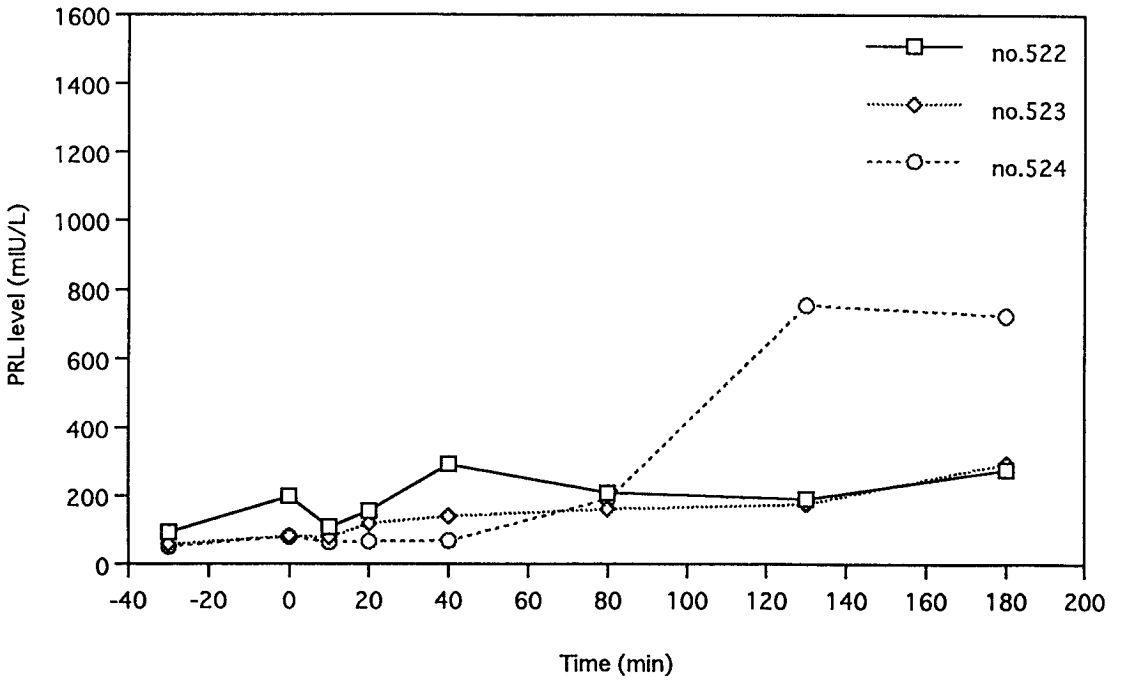

Fig. 9. Serum prolactin levels in pubertal male cynomolgus monkeys after intramuscular injection of 10 mg/kg ketamine at 0 minutes.

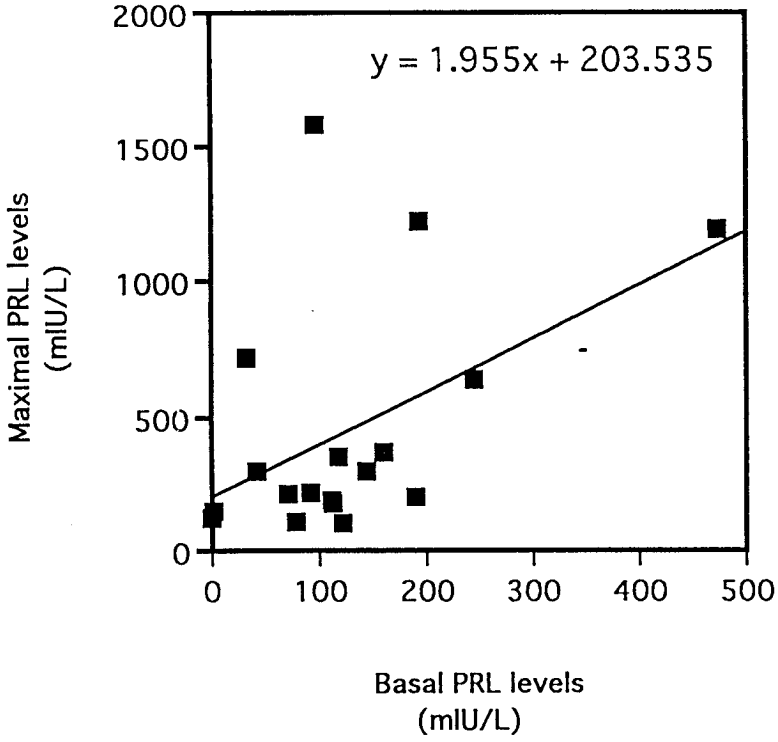

Fig.10. The relationship between maximal PRL levels and basal PRL levels in pubertal and adult male cynomolgus monkeys in response to 5 and 10 mg/kg ketamine injection.

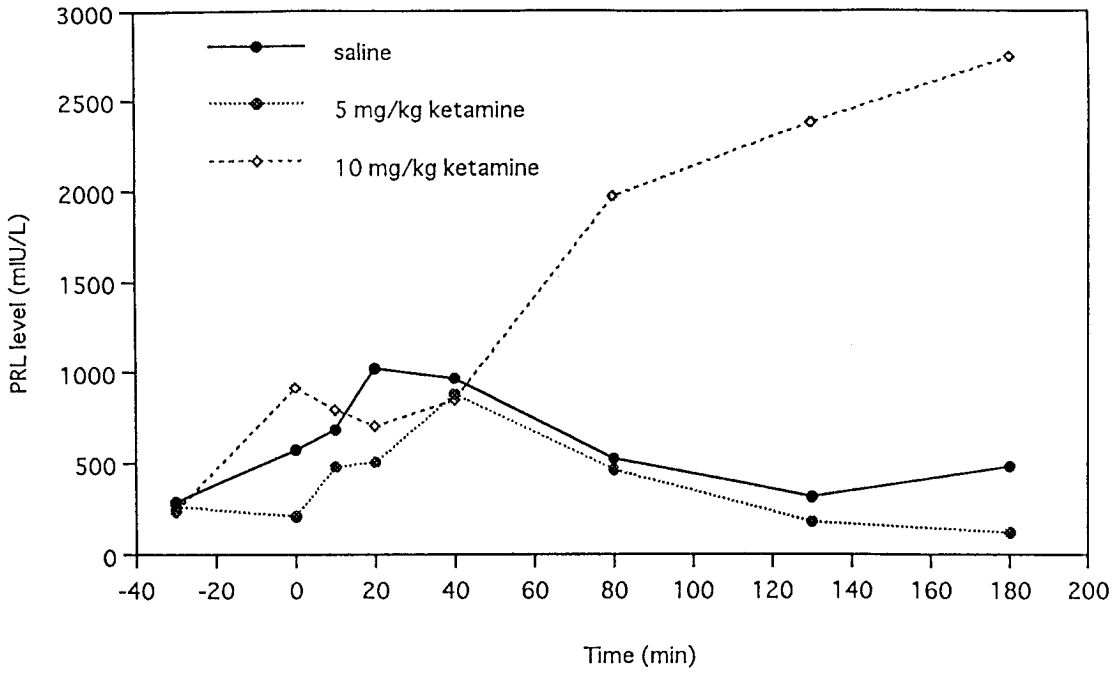

Fig. 11. Patterns of serum prolactin levels in male monkey (5 years old) which was postulated to expose to stress after saline, 5 mg/kg and 10 mg/kg ketamine injection.

The data of ketamine anesthesia in male cynomolgus monkeys in this study were in agreement with the previous reports from the other primate species[5,11,12] and in female cynomolgus monkeys[13,14] that ketamine hydrochloride could stimulate PRL release only in some monkeys. The implication of this finding for the different PRL response to ketamine is not clear. Since, the prominent changes did neither depend upon the age of monkey nor the dosage of ketamine treatment. However, monkeys having higher basal levels of PRL were seemingly responded to ketamine in a greater degree than others. On the other way, it is tempting to speculate that the stimulation of PRL release depends upon the hormonal pool.

The pattern of serum PRL levels after ketamine injection was nearly similar to the pattern from morphine (opiate agonist) injection which reached a maximum value at 30 minutes.[19] Smith *et al.* (1980)[20] and Finck and Ngai (1982)[21] reported that ketamine stereospecifically bound to opiate receptors in rat brain homogenate. In addition, the analgesic effect of ketamine was also inhibited by the opiate receptor antagonist naloxone. It may suggest that ketamine-induced PRL release in these male cynomolgus monkeys is at least partially related to the ability of the drug to act as an agonist at the opiate receptor in the central nervous system. Since there are a number of studies of PRL elevation after opiate administration in primate[22-27]. However, an effect of ketamine on PRL levels by other neuronal pathways is not excluded, since ketamine could induce PRL release only in some monkey as mentioned previously. Pekoe and Smith (1979)[28] found that the specific receptor blockers for neurotransmitter serotonin and norepinephrine attenuated the analgesic action of ketamine. Azzaro and Smith (1977)[29] and Smith (1977)[30] indicated that ketamine inhibited both dopamine and serotonin uptake and these two neurotransmitters have a profound effect on PRL secretion.

Many investigators studied a variety of stress effect on ACTH/cortisol in monkey and human.[31-33] The pituitary-adrenal-cortical activity was profoundly used to indicate stress situation. Actually, stress caused the release of immunoreactive CRF (ir-CRF) in several brain areas. The stress also caused a PRL increase[34], but the mechanism is still investigated. The monkey which was postulated to expose to stress responded to saline in the same pattern as 5 mg/kg ketamine injection. Therefore, it is difficult to assess that the augmentation of PRL levels after ketamine injection has come from the ketamine effect and/or stress of repeated venipuncture. Indeed, PRL level was increased even in monkey trained to offer his limb for venipuncture, although the mean PRL level was significantly less in the trained monkey than in the stress monkey.

Bearing this limitation in mind, the hormonal interpretation of PRL concentrations determined from the monkey that the experimental manipulations necessarily involve the use of either chemical or physical restraint procedure which may influence endocrine function should be carefully done. Nevertheless, the monkeys have already been trained to adapt to the experimental condition.

ACKNOWLEDGEMENTS

We would like to express our profoundly sincere gratitude to Dr.Takashi Yoshida at Tsukuba Primate Center for Medical Science, Japan, for his invaluable discussion of statistical analysis and parallelism check. Sincere appreciation is also extended to Professor Dr.Masumi Nozaki at Sado Marine Biological Station, Niigata University, Japan, for his grateful guidance. This work was supported by Chulalongkorn University Research Unit Grant and the National Research Council of Thailand.

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บทคัดย่อ

ลิงหางยาว (*Macaca fascicularis*) เป็นลิงที่พบมากในประเทศไทย จากขนาด รูปร่าง และน้ำหนักที่เหมาะสม จึงได้นำมาใช้เป็นสัตว์ทดลองทางวิทยาศาสตร์การแพทย์กันอย่างกว้างขวาง โดยเฉพาะอย่างยิ่งในกรณีที่การทดลองดังกล่าวไม่สามารถกระทำได้ในคน แต่เนื่องจากลิงหางยาวเพศผู้ค่อนข้างแข็งแรงและยากที่จะฝึกให้ยอมรับการทดลองได้ จึงต้องทำการสลบก่อนการทดลองเสมอ ยาสลบที่นิยมใช้กันทั่วไปคือเคตามีน มีรายงานจำนวนมากพบว่ายาสลบเคตามีนสามารถมีผลกระทบต่อระดับฮอร์โมน ดังนั้น ในการทดลองครั้งนี้ ได้ศึกษาผลของยาสลบเคตามีนในขนาด 5 และ 10 มก./กก. ที่มีต่อระดับฮอร์โมนโปรแลกตินในลิงหางยาวเพศผู้ จากหน่วยวิจัยไพรเมท คณะวิทยาศาสตร์ จุฬาฯ การตรวจวัดหาปริมาณฮอร์โมนโปรแลกตินในลิงหางยาว กระทำโดยวิธี RIA โดยใช้ commercial kit สำหรับตรวจวัดหาปริมาณฮอร์โมนโปรแลกตินในคน โดยตรวจสอบ parallelism ระหว่าง immunoreactivity ของ hPRL standard กับฮอร์โมนโปรแลกตินของลิงหางยาวที่สกัดได้จากต่อมใต้สมอง และจากการกระตุ้นด้วยฮอร์โมน thyrotropin releasing hormone (TRH)

จากการตรวจวัดหาปริมาณฮอร์โมนโปรแลกติน ที่เวลา -30, 0, 10, 20, 40, 80, 130 และ 180 นาที ภายหลังจากฉีดยาสลบเคตามีนเข้ากล้ามเนื้อ พบว่าระดับฮอร์โมนโปรแลกตินมักเพิ่มสูงขึ้นที่เวลา 40 นาที และลดลงสู่ระดับปกติในเวลาต่อมา ซึ่งการเปลี่ยนแปลงที่ชัดเจนของระดับฮอร์โมนโปรแลกตินพบในลิงบางตัวเท่านั้น โดยไม่สอดคล้องกับอายุของลิงและขนาดของยาที่ให้ แต่เป็นที่น่าสังเกตว่าลิงที่มีค่าปกติของระดับฮอร์โมนโปรแลกตินที่สูง จะมีการเพิ่มสูงขึ้นของระดับฮอร์โมนโปรแลกตินภายหลังจากได้รับยาสลบเคตามีนมากกว่า จึงอาจกล่าวได้ว่าความแตกต่างในการตอบสนองดังกล่าวนี้ขึ้นอยู่กับ hormonal pool ที่ต่อมใต้สมองส่วนหน้าของลิงแต่ละตัว ดังนั้น ในการวิเคราะห์ค่าปริมาณฮอร์โมนโปรแลกตินในลิงหางยาวเพศผู้ที่ทำการสลบก่อนเจาะเลือดด้วยยาสลบเคตามีน ควรจะต้องทำด้วยความระมัดระวัง