# CHAPTER V

# DISCUSSION

## Acute Effect of Morphine Hydrochloride

Although chronic studies are often useful to describe the overall hormonal profile in an addicted patient and animal chronically exposed to narcotics. Chronic drug administration provides a very poor vehicle with which to study drug-specific effects. Therefore, after a period of chronic drug exposure, one has no idea whether a drug is exerting a specific effect of its own on a certain hormone or if what one is observing is a secondary, compensatory alteration in that hormone resulting from a drug effect at an entirely different site. Finally, chronic drug administration can produce a physiological change or organ pathology, any of which could cloud the interpretation of endocrine data. For these reasons, acute drug studies will be profitable. In this study, the effect of a single subcutaneous morphine administration on PRL, testosterone, TSH, $T_4$ and cortisol will be discussed.

### 1. The effect on PRL levels

As in a number of studies (Tolis, Hicky and Guyda, 1975; Rivier et al., 1977; Gold, Redmond and Donabedian, 1979; Wehrenberg et al., 1981; Spiegel, Kourides and Pasternak, 1982), PRL levels occasionally peaked at 30 minutes after morphine administration. In

addition, the PRL response to morphine in these male cynomolgus monkeys was not different from female cynomolgus monkeys (Setheetham, 1992) in which the levels of PRL rise were particularly a dose-related manner. The mechanism of morphine acted as a stimulator for PRL secretion rather mention about hypothalamic level, since opiates did not induce PRL release when added directly into pituitary glands culture (Rivier et al., 1977) or failed to cause any PRL increase in pituitary stalk-section monkeys (Wardlaw et al., 1980; Wehrenberg et al., 1981). Opiates inhibited the synthesis (Alper, Demarest and Moore, 1980), release (Gudelsky and Porter, 1979) and turnover (Deyo, Swift and Miller, 1979; Alper, Demarest and Moore, 1980) of dopamine from the tuberoinfundibular dopaminergic (TIDA) neurons which in turn would increase PRL release from the anterior pituitary. However, Enjalbert and his colleagues (1979) found that morphine could modulate the inhibitory effect of dopamine on PRL release directly at the pituitary gland. In case of a rapid rise in PRL levels after morphine administration, the other neuronal pathways e.g., prolactin-releasing factors (PRF) were included to explain this regulation. Van Vugt and Meites (1980) suggested that opiates exerted their PRL-releasing effects via mediation of serotonergic system, since the administration of serotonin receptor blocker, metergoline and methysergide, significantly reduced the PRL-releasing effect of Met-Enk in the rat (Spampinato et al., 1979). Hence, the opioid pathways regulating PRL secretion occasionally acted through serotonergic as well as dopaminergic mechanism. In addition, TRH, an outstanding PRF, potentiated the PRL release by an effect of endogenous opiate (Buydens et al., 1987).

Pubertal male monkeys injected with 3.0 mg/kg morphine hydrochloride produced a lesser extent in PRL response than adult male monkeys that recieved the same dose of morphine. Spiegel et al. (1982) accurately claimed that $u_1$-subtype of opiate receptors contributed to PRL secretion. The finding in the rat that a serotonin-independent u-receptor-mediated control of PRL secretion could be stimulated early in ontogeny, whereas a serotonin-mediated u control of PRL secretion that developed by 20-day old (Blackford et al., 1992). It may suggest that in pubertal age of cynomolgus monkey a serotonin-mediated u control of PRL secretion has not been develop yet. That is adult monkeys represent a greater degree in PRL secretion during morphine treatment than pubertal monkeys by influencing both dopaminergic and serotonergic systems.

Since serum PRL concentration showed a marked nyctohemeral rhythm with high levels during the night and low levels during the day (Quadri and Spies, 1976). In rhesus monkey, PRL levels were increased markedly by 2000h under the 4 hours bleeding interval (Quadri and Spies, 1976). With regard to this study, the acute effect of morphine on PRL levels was taken during 0700-1900 h. Hence, in my hands, the sustained PRL rise higher than basal levels at the end of study period or at 1900 h could not postulate that these results were produced by high doses of morphine using in this study or the effect of nyctohemeral rhythm. On the previous study in *Macaca arctoides* showed that an injection of morphine intravenously produced a maximal PRL level at 45 minutes and subsided to normal levels approximately at 3 hours after an injection (Gold, Redmond and Donabedian, 1979). On a practical level, the study was designing

experiments and interpreting data that included prolactin measurement over long intervals should be suggested a caution.

2. The effect on testosterone levels

Data in basal testosterone levels, including adult and pubertal male cynomolgus monkeys, are in general agreement with those of others male cynomolgus monkey (8.7 ± 1.5 ng/ml; Adams et al., 1988), rhesus monkey (5.0 ± 0.8 ng/ml; Goodman et al., 1974) and man (4.6-6.2 ng/ml; de Lacerda et al., 1973; Rosenfield, Jones and Fang, 1977). Changes in testosterone levels after morphine administration in male cynomolgus monkeys occurred in the face of seemingly the same as in the rat (Cicero, Meyer et al., 1976; Cicero, Bell et al., 1977b), rhesus monkey (Gilbeau et al., 1984) and man (Mendelson and Mello,1975). Serum testosterone levels in morphine treated monkeys declined significantly in a dose-dependent manner. The higher dose treatment could decrease testosterone levels rapidly and protracted to the end of the study period.

Morphine acutely depressed serum testosterone levels, thus, the direct inhibition of testicular steroidogenesis and enhancement of the degradation of testosterone were suggested (Tsong et al., 1982; Tsong, Phillips et al., 1982; Chen et al., 1984, Gerendai, 1991; Chandrashekar and Bartke, 1992). Nevertheless, numerous reports indicated that neither acute nor chronic morphine administration enhanced the catabolism of testosterone by the liver or in any way influenced the uptake, metabolism, or biochemical effects of testosterone in any of its known target organs (Cicero et al., 1976; Cicero, Badger et al., 1977; Cicero, Bell et al., 1977).

Moreover, morphine did not adversely affect the function of the secondary sex organs in hypophysectomized rat in which normal testicular function was restored by treatment with hCG (Cicero, Meyer et al., 1976) or lacked of an effect on androgen production in hCG pretreated monkeys (Gilbeau et al., 1984). Available evidences therefore suggested a direct action of opiate on the hypothalamic-pituitary-gonadal axis. Male rats subcutaneously injected with morphine could promptly lowered serum LH levels within 30-40 minutes, reaching a peak of depression at 1 hour of post-injection (Cicero et al., 1975; Cicero, Meyer et al., 1976; Cicero, Badger et al., 1977; Cicero, Bell et al., 1977). These times corresponded very closely with the peak serum morphine levels which occurred approximately 15 minutes before LH decrease (Cicero, Bell et al., 1977). Naloxone treatment resulted in an increase in LH levels at the corresponding time (Morley et al, 1980; Gosselin et al.,1983; Gilbeau et al., 1984). The drop in LH levels preceded by more than 1-2 hours a fall in testosterone levels (Cicero, Bell et al., 1977).

Morphine was not significantly modified the stimulation of LHRH on LH release by the rat pituitary *in vivo* and *in vitro*. Similarly, chronic morphine administration significantly increased the levels of LH in the pituitary, while at the same time depressing serum levels of LH (Cicero, Badger etal., 1977). Electrical stimulation of the median eminence also blocked the antiovulatory action of morphine, suggesting that morphine particularly inhibited the release, but not necessarily the synthesis, of LHRH (Van Vugt and Meites, 1980).

From the hormonal profile in cynomolgus monkey (Varavudhi, Tangpraprutigul and Asawaroengchai, 1982) as in rhesus monkey (Goodman et al., 1974), the diurnal variation in testosterone levels showed a peak at 2100 h with the begining of rise at 1800 h and a nadir at 0900 h. In this study, blood samples were taken during 0700-1900 h. Therefore, at the end of the study onset, testosterone levels were observed to increase, particularly in the low-dose treatment. Controversy, in the high-dose treatment testosterone levels were still decline, since morphine effect marked the diurnal variation phenomena. At the same dose of morphine treatment 3.0 mg/kg, the effect on serum testosterone levels appeared to be dependent upon the age of monkeys. That is, serum testosterone levels were reduced slower and in longer time in pubertal males than were in adult males. As compared with the rat, testosterone levels also decreased slowly with a greater degree in younger rats than they were in adult rats (Cicero, Meyer et al., 1976). In keeping with this data, the testosterone levels in pubertal control after saline-injection are very fluctuation. However, the hormonal pattern after saline-injection can be discriminated from the pattern in morphine response. That is, testosterone level reaches a nadir at 2.5 hours after saline injection and gradually increase thereafter, but it gradually decreases and subsides at 10 hours after morphine injection. Therefore, it should be born in mild for the single blood collection and data interpretation in long-term study of young-age monkey.

3. The effect on cortisol levels

All male cynomolgus monkeys displayed the early morning rise and late evening fall of serum cortisol levels. The episodic secretion of cortisol in this macaque species are in agreement with the pattern reported in human (Dent et al., 1981; Grossman et al., 1982), but distinguished from nocturnal mammals as the rat (Kiem et al.,1987; Kant, Mougeyand Meyerhoff,1986). These rhythms are generally entrained to the sleep-wake or activity cycle of animals. Levels of adrenocorticoid reach their peaks just before the onset of awake phase. In man, cortisol levels and the fequency of secretory episodes peak just prior to awaking (Kreiger, 1979). In the nocturnal animals, levels of corticosterone rise during the latter part of light period and peak prior to the onset of darkness (Kant, Mougey and Meyerhoff, 1986). It may come from this reason, morphine administration caused a suppression of cortisol levels in all male cynomolgus monkeys as in gilts (Barb et al., 1986; Estienne et al., 1988) and human (George et al., 1974) but vice versa from the rat (Buckingham et al., 1982). Therefore, the document of morphine effect on cortisol levels in male cynomolgus monkeys may be capable of trasferring to human subjects.

Nearly all monkeys, the cortisol levels were particularly high during the time of morphine injected (time 0). Since, animals were anaesthetized with ketamine hydrochloride preceding the first blood sample collection. Therefore, blood samples collected at -60 and -30 minutes were from unconscious monkeys. Although ketamine itself did not affect basal cortisol levels in cynomolgus monkeys (Castro et al., 1981), but the pituitary-adrenal axis could

significantly activated in monkeys unaccustomed to routine handling (Puri, Puri and Anand Kumar, 1981). The dose of ketamine used in this study was the minimum effective dose needed for temporary immobilization only. All monkeys could recover and became alert in a shorter duration (approximately 20 minutes) than the other report (30 minutes) in rhesus monkeys (Ochsner, 1977). Hence, the blood obtained during the first hour after morphine injection were withdrawn from monkeys in conscious state. It is possible that a result of stress induced by blood sampling and handling conscious monkeys as evidenced in chimpanzee monkeys causes a rise in serum cortisol levels (Gosselin et al., 1983).

It was previously shown that a short-time noxious foot-shock stress caused a depletion of B-EP in the hypothalamus, septum and periaqueductal grey, indicating an enhanced release of the peptide therein (Millan et al., 1981; Przewlocki et al., 1982). More recently, it was shown that acute swim stress decreased the B-EP content in the nucleus accumbens septi, again suggesting an accelerated release of this peptide from nerve terminals (Przewlocki et al., 1989). Immunoreactive B-EP has been detected in human plasma and has been shown to be secreted from the pituitary gland concomitantly with ACTH and other peptide fragments of POMC (Wardlaw and Frantz, 1979). Also B-EP and ACTH were simultaneous increase before and during physical exercise that has been viewed in the broader context of physical and psychological stress (Oltras, Mora and Vives, 1987). It resulted in the consequent increase in the cortisol levels (George et al., 1974).

Since morphine, an exogenous opiate, did not affect the normal adrenal response to exogenous ACTH, the evidently proposed at the hypothalamic-pituitary level (George et al., 1974). Similarly an B-EP, endogenous opiate, was known to decrease pituitary secretion of ACTH and, thus, could potentially regulate cortisol output indirectly (Beyer et al., 1986). Estienne et al.(1988) found that the increase in serum cortisol concentrations following naloxone administration to female pigs was abolished by hypophysial stalk-transection, even though CRH and ACTH stimulated cortisol release in these animals. It means that endogenous opioids decrease cortisol secretion primarily at hypothalamic level. Others have demonstrated that opioid antagonism may enhance cortisol secretion by acting either on the hypothalamus to augment release of CRH (Eisenberg, 1984) or on the pituitary gland to cause release of ACTH (Volavka et al., 1979; Siegel et al., 1982; Jezova, 1985; Grossman et al., 1986). Taken together, these observations suggest that an inhibitory opioid pathway is involved in the basal regulation of ACTH and this inhibition is unmaked by naloxone. However, a direct effect of opiates on the adrenal gland has not yet been rule out owing to the integrated cortisol responses to the bolus dose of ACTH when preceded by a high pharmacological dose of B-EP were significantly lessened (Beyer et al., 1986).

Following saline injection, there was a gradual fall in circulating cortisol in keeping with its known circadian rhythm. Morphine produced a marked decrease in cortisol and the low level was greater than following saline at 2.5 hours. Of interest, the lower dose of morphine produced a more significant reduction in

cortisol levels. Surprisingly, cortisol levels in adult monkeys treated with 6.0 mg/kg morphine hydrochloride always showed no statistically significant from the saline-injected control. Buckingham and Hodges (1979) also found that the secretion *in vitro* of rat hypothalamic CRH was less marked in higher concentration of morphine as compared to production in low concentration. This may possibly due to the fast, rate-sensitive feedback mechanism of hypothalamic-pituitary-adrenal activity mediated by circulating corticosteroids (Jones, Hillhouse and Burden, 1977). Gibson et al. (1979) reported that the plasma corticosteroid response to ether stress was reduced by a preceding stress. Futhermore, Buckingham (1979) has shown that the feedback mechanism operated by corticosteroids was not appearent until 1 hour later. In this study morphine in dose 6.0 mg/kg unable to decrease serum cortisol levels even earlier than 1 hour after administration. Thus the feedback mechanism operated by morphine may not mainly due to circulating corticosteroids but may likely due to interaction of morphine with inhibitory noradrenergic neurons in the hypothalamic-pituitary-adrenal system (Buckingham and Hodges, 1978). Since, the pretreatment with the noradrenergic alpha-1 antagonist, thymoxamine, also blocked a rise in plasma cortisol after naloxone injection (Grossman and Besser, 1982). By nature, two out of three monkeys (no.506 and no.704) treated with 6.0 mg/kg morphine displayed a very aggressive behavior. They did not habituate to the handling, therefore, they may feel stress during the blood collection. Shively and Kaplan (1984) represented the case in male cynomolgus monkeys that increased levels of adrenal cortical activity and hypertrophy of adrenocortical tissues to defeat in aggressive encounters and to

conditions of social subordination were in general. This may reflect on opioid-mediated inhibitory pathways which were already maximally operative and, therefore, not responsive to exogenous opioid administration as morphine (Gosselin et al., 1983). Additionally, endogenous opiate produces a constant tonic inhibition on pituitary-adrenal axis throughout 24 hours (Grossman et al., 1982). Thus morphine administration can potentiate, at least in part, an endogenous endorphin to decrease cortisol levels which can recover to the normal circadian variation thereafter.

4. The effect on TSH levels

The result of morphine effect on TSH levels in male cynomolgus monkeys was similar to the effect reported in human as cortisol hormone was. That is, morphine showed either no effect on TSH levels (Tolis, Hicky and Guyda, 1975; Morley et al., 1980; Reid et al., 1981) or stimulated TSH release (Stubbs et al., 1978; Delitala, Grossman and Besser, 1981). But it has been conflicting with the reports in rats that morphine decreased basal TSH or cold-stimulated TSH levels (Muraki et al., 1980; Sharp et al., 1981;Judd and Hedge, 1982; Mannisto et al., 1984; Arancibia et al., 1985; Rauhala, Mannisto and Tuominen, 1988; Berglund et al., 1990; Dou and Tang, 1993). In this study, morphine in the lowest dose (1.5 mg/kg) stimulated the secretion of TSH while in higher doses (3.0 and 6.0 mg/kg) without affecting. When compared with the saline control, a significant change was also typically observed only in the lowest dose treatment and in pubertal monkeys. This evaluation of serum TSH suggested that it did not produce by a circadian variation or stress as in the other hormones said before. Since TSH levels showed a

circadian rhythm with levels rising in the evening (Hershman, 1980). A consistent TSH surge occurred in advance of the onset of sleep and peaked at 2300 h with the duration of 4-6 hours in man (Vanhaelst et al., 1972; Azukizawa et al., 1976; Greenspan et al., 1986). But TSH rise in this study was found suddenly 15 minutes after 1.5 mg/kg morphine administration (within 0800-0900 h). Additionally, this TSH alteration did not result in stress effect, since stress has reduced serum TSH levels rather than increase (Sower et al., 1977; Sharp et al., 1981; Judd and Hedge, 1982), possibly due to increased cortisol secretion (Ostuki, Dakoda and Baba, 1973; Sower et al., 1977; Pamenter and Hedge, 1980).

Sawin and Hershman(1976) and Sawin et al.(1978) reported in man that baseline serum TSH levels have strongly correlation with the peak response of serum TSH after TRH. In this study, three out of four monkeys treated with 1.5 mg/kg morphine have basal TSH levels higher than the other monkeys, then, it may cause a profound increase in TSH levels after morphine injection in this monkey group. Such finding imply that morphine stimulates TSH secretion via a hypothalamic action on TRH release. A number of literatures showed that morphine affected TSH secretion when microinjected into the hypothalamus (Judd and Hedge, 1982; Mannisto et al., 1984; Arancibia et al., 1985) of central sites (Muraki et al., 1980) but did not attenuate the TSH release induced by TRH (Muraki et al., 1980). In pituitary culture, the addition of opioid peptides did not modify TSH release (Jordan et al., 1986). If morphine could stimulate TSH release directly at pituitary level, the higher dose of morphine treatment (6.0 mg/kg) in this study should produce a greater degree

of TSH elevation rather than reduction.

Since TSH and its two subunits secretion and synthesis are principally regulated by TRH and dopamine (Azukizawa et al., 1976; Sawin and Hershman, 1976; Burrow et al., 1977; Shupnik, Greenspan and Ridgway , 1986). Therefore, morphine modulated TSH release may also invole in dopaminergic mechanism. Dopamine infusion in normal males obliterated and markedly attenuated the mean peak serum TSH and PRL concentrations in responseto TRH (Burrow et al., 1977). Morphine profoundly increased PRL levels (Gold, Redmond and Donabedian, 1979) and was thus implicated in the decrease of dopamine synthesis (Alper, Demarest and Moore, 1980), release (Gudelsky and Porter, 1979) and turnover (Deyo, Swift and Miller, 1979) from TIDA neurons. In addition, the degree of TSH release after the dopamine receptor blocking drug, metoclopramide administration was inversely related with the basal levels further support the possibility of dopamine as a dominant factor affecting lower daytime TSH levels (Scanlon et al., 1979). Hence, in monkey groups treated with higher doses (3.0 and 6.0 mg/kg) of morphine hydrochloride which were obligated with low basal TSH levels should be more sensitive to the suppression by dopamine and would simultaneously inert to the stimulation of TRH. It was then rarely to observe a significant increase of serum TSH levels after morphine administration. Additionally, one of adult male monkey treated with 6.0 mg/kg morphine hydrochloride (no.508) died at 150 minutes of successive blood collection of this study period. The animal numbers were reduced thereafter and could not be represented the significant effect of morphine on TSH levels.

The implication of this finding for the different TSH response to morphine is unclear. It may be tempting to speculate that this dose-related response in TSH levels induced by morphine were the role of multiple opiate receptors. Morphine, the most selective u agonist, increased both PRL and GH levels (Rivier et al., 1977; Sharr and Clemens, 1980). Maximum PRL release required lower dose of morphine than those needed for the maximum GH response and naloxazone, selective blockade of $u_1$ (high affinity) opiate receptor, reduced morphine-induced peak concentrations of PRL only (Spiegel, Kourides and Pasternak, 1982). These results imply that morphine-induced GH release were modulate through a lower affinity receptor (kappa receptor) whereas at higher dose of morphine could interact (Penchnick, George and Poland , 1985). With respect to TSH release, DAMME a long-acting analogue of met-enkephalin produced a clear elevation in TSH levels (Stubbs et al., 1978; Delita, Grossman and Besser, 1981) while morphine notably had no effect (Reid et al., 1981). DAMME was primarily binding to u-receptor and deprivation of binding affinity to K-receptor (Kosterlitz, 1991). These data may compatible with the hypothesis that morphine could interfere with two differences, but inter-dependent receptors: at low dose morphine could act at one receptor site (u-receptor) to cause an increase of serum TSH, whereas at higher dose it could interact with a second site of lower affinity (K-receptor) that is responsible for the inhibition of TSH secretion. When morphine(at high dose) activates the lower affinity site, the interaction with this receptor counteracts and reverses the effects of the high affinity site (Amoroso et al., 1988), then no alteration in serum TSH levels could be observed thereafter. On the basis of this hypothesis naloxone can

block both receptors (Gilman et al., 1985)

Since the administration of naloxone alone was unable to alter serum TSH levels (Morley et al., 1980). This may suggest that endogenous opiate-like peptides might have no physiological role in modulating the TSH level. Furthermore, morphine attenuated a cold-stimulated and heat- or stress-reduced TSH secretion after both peripheral and intracerebroventricular administration in the rat (Muraki et al., 1980; Sharp et al., 1981). From these reasons, it may conclude that morphine is without an effect on basal TSH levels but may act on stress-induced TSH secretion. Finally, the preliminary study on long-term treatment of 0.1-0.8 mg/kg/day morphine hydrochloride in female cynomolgus monkeys could not be found the notably alteration in serum TSH levels, even the treatment was long as 100 days (Malaivijitnond, 1990)

5. The effects on $T_4$ levels

The basal serum $T_4$ levels of male cynomolgus monkeys in the present study are in agreement with the previous reports in human (7.3 $\pm$ 1.5 ug/dl, Azukizawa, Pekary et al., 1976; 7.4 $\pm$ 1.6 ug/dl, Greenspan et al., 1986), rhesus monkeys (6.0 $\pm$ 1.3 ug/dl, Azukizawa, Murata et al., 1976) and female cynomolgus monkeys (6.20 $\pm$ 0.17 ug/dl, Malaivijitnond, 1990). The basal $T_4$ levels were without a sex different whereas TSH levels in human female was higher than in male (Sawin and Hershman, 1976; Sawin et al., 1978). On the previous days, the published reports about the acute effect of morphine on $T_4$ levels were done in rats only (Lomax and George, 1966; Lomax, Kokka and George,1970; Tal et al., 1984; Tal et al.,

1986 Bhargava et al., 1989; Berglund et al., 1990, del Valle-Soto et al., 1991). Microinjection into the hypothalamic site or intraperitoneal injection of morphine into the rat depressed the release of radioiodine from the thyroid gland (Lomax and George, 1966; Lomax, Kokka and George, 1970). Lomax, Kokka and George (1970) suggested a dual action of morphine : stimulation of neurons in the caudal hypothalamus that inhibited TSH release and depression of neurons in the rostral region that normally activated the pituitary-thyroid axis. Morphine caused a decline of basal and cold-stimulated TSH levels in the rat via TRH system (Muraki et al., 1980; Judd and Hedge, 1982; Ruzsas and Mess, 1983; Berglund et al., 1990). Since pretreatment with morphine did not reduce the release of TSH induced by exogenous TRH (Muraki et al., 1980). Therfore, it was actually followed by a decrease of $T_4$ levels (Berglund et al., 1990). However, the direct effect of morphine on the thyroid gland was also evidenced (Tal et al., 1986). Freire-Garabal et al.(1992) reported opioid binding sites in the thyroid gland, and the male rat thyroid gland incubated with morphine *in vitro* produced a significant increase in $T_4$ concentration of incubation medium (Tal et al., 1986).

In contrast to the rat, morphine induced (Delita, Grossman and Besser, 1981) or without (Reid et al., 1981) an effect on TSH levels in human which was similar to in cynomolgus monkeys. This should be coincided with an increment of $T_4$ levels, since a significant rise of plasma $T_3$ and $T_4$ levels from the basal values were observed at 30 and 45 minutes, respectively, in the female rhesus monkeys preceding with the rise of plasma TSH levels at 15 minutes after TRH injection (Azukizawa, Murata et al., 1976).

Ho et al.(1977) determined $T_4$ levels in chronic heroin addicts, the average length of addiction was 10 years, and reported a higher $T_4$ levels than that of the normal subjects. In agreement with this study, this increment of $T_4$ levels may result in the peripheral effect of morphine. Since it was associated with a lower cholesterol level and accounted for by thyroxine-stimulated increase of cholesterol metabolism which was a prerequisite of metabolic rate controlling. Consequently, Ho et al.(1977) assumed that the increase of $T_4$ level, in this particular case, may produce via the stimulated TSH secretion.

In this acute study, TSH levels promtly increased after morphine injection, particularly in adult monkeys treated with 1.5 mg/kg morphine. The significant increase of TSH began at 15 minutes and followed by the $T_4$ rise at 150 minutes. In the remain groups, TSH levels were also increased nearly at the last point of successive blood collection (within 270-390 minutes) and simultaneously with the cascade rise of $T_4$ concentrations. Therefore, it may suggest that effect of morphine on serum $T_4$ levels in male cynomolgus monkey was different from the effect found in rats (Bhargava et al., 1989; Berglund et al., 1990), although it may resemble on TSH and cortisol responses. However, the increments of TSH and $T_4$ in this onset of study were in the normal fluctuation ranges, because the saline control by itself showed an increment of $T_4$ pattern nearly identical to the response of morphine treated animals. Azukizawa, Pekary et al.(1976) suggested in human that the short-term elevation of serum $T_4$ may result from changes in protein concentration due to hemodynamic response to

alteration of physical activity and not TSH rise.

The Acute Effect of Morphine Hydrochloride on Hormonal Levels in Naive Monkeys

The study of acute effect of morphine hydrochloride on hormonal levels (TSH, $T_4$, cortisol, PRL and testosterone) in naive monkeys were similar to the recovered morphine-addiction monkeys. There were using only 2 to 3 experimental monkeys in this study, then, the standard deviation of mean was rather high. Bearing this limitation in mind, the statistical difference by unpair t-test could not sometimes obtain. As mentioned previously, these animals were never collected the blood sample before, therefore, they were precipitated in the stress during studied period. Stress is a particular factor in stimulating cortisol release (Rossier et al., 1977; Christy, 1978). Therefore, the cortisol levels at 0 minute were significantly higher than at -60 minutes.

It may assume that morphine addicts resulted in hormonal alteration could be recovered to a normal endocrinological status in a short-duration. After that, they can response to the narcotic drug in hormonal changes again as prior to using the drug.

In sum, the study of acute effect of morphine hydrochloride on any hormonal changes in male cynomolgus monkeys was concurred with in human but partly did so in the rat. For example, the reduction in cortisol levels (George et al., 1974) and the elevation in TSH and $T_4$ levels in male cynomolgus monkey were resemble with human (Tolis,

Hicky and Guyda, 1975; Stubbs et al., 1978;Morley et al., 1980; Delitala, Grossman and Besser, 1981; Reid et al., 1981) but vice versa from rats (Muraki et al., 1980; Sharp et al., 1981; Buckingham et al., 1982; Juud and Hedge, 1982; Mannisto et al., 1984; Arancibia et al., 1985; Berglund et al., 1990; Dou and Tang, 1993). It may resulted from the reason that monkey and human are the diurnal animal whereas rat is the nocturnal animal (Grossman et al., 1982; Keim et al., 1987). They have a sleep-wake cycle in the opposite site with consequently are so in their daily activity (Kant, Mougey and Meyerhoff, 1986). Therefore, the diurnal variation of cortisol, TSH and $T_4$ levels which are important hormones related to metabolic rate (Smith et al., 1983) are in different patterns. Usually these hormones are peak just prior to the onset of awake phase (Kant, Mougey and Meyerhoff, 1986). As in these reasons, the hormonal alterations after morphine treatment are distinguish. Therefore, the information in these male cynomolgus monkeys should be useful for clinical evaluation in human. Since, we can sometimes not study the effect of morphine in human that was concreted by moral and ethical standards.

Chronic Effect of Morphine Hydrochloride

Clinically, chronic addiction to opiate drugs can lead to a number of physiological and psychlological disturbances that will ultimately affect the general health of the addicted subject. The bases of such disturbances are not well understood. It is generally believed that any physiological consequences of opiate addiction must first be mediated through the binding of the opiate drug to

specific receptor sites in the central nervous system (Snyder, 1975). Disturbances of endocrine functions have been proposed as one of the causes for various physiopsychlological changes during chronic opiate addiction. The most notable example of this is the reduction of sex drive in male addicts; the cause of this has been suggested with a decrease in testosterone level (Cushman, 1972, 1973; Martin, 1973, Mandelson and Mello, 1975). To evaluate the role of morphine addiction for endocrine disorders the serum levels of PRL, TSH, $T_4$, $E_2$, testosterone and cortisol in male cynomolgus monkeys were chosen for determination. Notably, the hormonal pattern in this study were determined approximately 20 hours after daily single subcutaneous morphine injections as previously described in the materials and methods. Since the elimination half life ($t_{1/2B}$) of morphine in these male monkeys is 308.21 $\pm$ 20.3 minutes and approximately 90 percent was eliminated from the body fate within 24 hours (Wood and Wood, 1982). Then the onset of morphine effect on hormonal levels in the present study should represent the cascade of prolonged effect (20 hours) of morphine after binding to the opiate receptor or a sudden tolerance (adjustment) and dependence symptoms after a daily single morphine injections. For convenience, the mean hormonal levels in each monkey group responsed to each dose of morphine hydrochloride will be first discussed.

Based upon the long interval of the blood collection after morphine injection, the effect of morphine on the hormonal alterations can not be observed prominently. In the acute effect study, morphine decreased the cortisol levels at 2.5 hours and recovered to the normal circadian variation, thereafter. As in the

human, subcutaneous administration of morphine sulfate resulted in the lowered hydrocortisone level at 4 and 5 hours after the drug injection (McDonald et al., 1959). In addition, the lowered cortisol levels could return toward control levels 90-120 minutes after the termination of B-EP infusion (Taylor et al., 1983). However, the suppressive effect of morphine on cortisol level could still be found in the moderate dose of morphine (3.0 mg/kg/day) given to adult monkeys. While in the higher dose (6.0 mg/kg/day), the mean cortisol level during treatment period trended to be higher than pretreatment values, suggesting the mild withdrawal symptoms may appear during the single daily injection of morphine prior to recieving the next dose. As confirmed these results, the cortisol levels during the late treatment period trended to be higher than during the early treatment period. In addicted subjects the morning plasma levels of 17-hydroxycorticosteriod were at least twice as low during the addiction period as during the control period, but these patients recieved morphine (3.5 mg/kg) 4 times a day (Eisenman, Fraser and Brooks, 1961). Interestingly, during withdrawal period monkeys injected with highest dose of morphine hydrochloride (6.0 mg/kg/day) showed a sudden peak in mean cortisol levels following with an abrupt drop in the next week of blood collection. Eisenman, Fraser and Broks (1961) found that the time when maximal 17-hydroxycorticosteroid in urine was observed varied from 24 to 48 hours after withdrawal of morphine. However, the elevated levels of urinary 17-hydroxycorticosteroid did not return to normal for several days (Eisenman, Fraser and Brooks, 1961). Since, the last dose of morphine injection (6.0mg/kg/day) in these male monkeys was day-140 (at 1200-1300 h) and the first blood collection during

withdrawal period was day-142 (at 0800-0900 h). Therefore, the total interval time was 44 hours which was in the range of duration of sharply peak occurred. In the present study, It may conclude in addition to Eisenman et al.(1961) that the time of maximal cortisol occurred did not related to the duration of addiction but depended upon the doses of morphine administration. Since the higher doses of morphine administration, the shorter time for maximal cortisol levels were occurred. Whereas, the levels of cortisol rise were so depended upon the duration of addiction.

There was no discernibly affected of morphine on testosterone levels in this study. In agreement with Cushman(1973) and Celani et al.(1984) found that testosterone values of male heroin addicts were undifferent from that of non-addict control. This was particularly true in the case of acute effect study, morphine reduced testosterone levels until at 10 hours (or last point of blood collection) in a dose-dependent manner with gradually increase to the normal level afterward. The mean testosterone levels were considerably fluctuated during treatment and withdrawal periods in pubertal monkeys. It seem to be rebound phenomena upon morphine withdrawal after each morphine injection, and on ultimate adapted to normal levels. Accordingly, during posttreatment period pubertal monkeys exhibited a protracted rise in testosterone levels.

During precipitated morphine withdrawal, noradrenergic neurons in the locus coeruleus of the rat increased their activity (Crawley, Laverty and Roth, 1979; Gabriel, Simpkins and Millard, 1985). The brain noradrenergic system is stimulatory to LH secretion

(Gabriel, Simpkins and Millard , 1985). Therefore, the initial increases in norepinephrine turnover was associated with a marked increase in LH level during withdrawal period in rats (Simpkins, Katovich and Song, 1983). Moreover, the serum testosterone and LH levels in male rats returned to the control levels 48 hours after the withdrawal of morphine (Muraki et al., 1978; Cicero et al., 1983). It has been conflicting to the preceded results in rats. During withdrawal period testosterone levels in male cynomolgus monkeys were decreased, but this lowered levels were coupled with the elevation of cortisol levels. Since cortisol and synthetic analogue with strong glucocorticoid activity, dexamethasone are capable of inhibiting hCG-stimulated testosterone production via a glucocorticoid receptors on testicular interstitial cells *in vitro* and the effect was reversed by coincubation with antiglucocorticoid, RU486 (Urban, Miller and Dorsa, 1991; Orr and Mann, 1992). Thus, it provides insight how stress may affect testosterone production and reproductive organs. In addition, the administration of dexamethasone to male fetus rats at term induced 1 hour later a slight increase hypothalamic GnRH and pituitary LH content, reduced drastically plasma LH levels and completely prevented the postnatal testosterone surge which occurred normally in littermate controls (Lalau et al., 1990). From this reason, the reduction of testosterone levels mediated by cortisol may override the withdrawal effect of morphine which increased testosterone levels through noradrenergic system that occurred in a short-duration. Again, the duration of first blood collection in this study within 44 to 68 hours after morphine withdrawal may sufficient to produce a readjustment of testosterone levels to the normal

pretreatment values, then, the increment could not be found.

Little informations have heretofore been available about the effect of opiates on $E_2$ in man. A substantial portion of $E_2$ in male is derived by biosynthesis in the testes from testosterone, and the remainder is obtained from peripheral conversion of the same precursor (Kelch et al., 1972; Franz and Longcope, 1979; Prior et al., 1987). In addition, the conversion of plasma androstendione to estrone was progressively increase with an advancing age (Hemsell et al., 1974). Despite this relationship, the plasma $E_2$ concentrations of male opiate addicts did not different consistently from those of control (Azizi, Vagenakis and Longcope, 1973) whereas the reported testosterone concentration were lowered than normal values (James, Heywood and Crook , 1980; Malik et al., 1992). However, the normally low content of this hormone in male plasma (20 $\pm$ 1.6 pg/ml in human, 17 $\pm$ 5.7 pg/ml in rhesus monkey: Kelch et al., 1972 and 40-100 pg/ml in cynomolgus monkey: Meusy-Dessole and Dang, 1985), together with the possibility of episodic changes in its concentration present difficulties in using these criteria as a measure of changes in endogenous estrogen secretion. In compatible with these reasons, the alteration in $E_2$ levels could not detect in the present study and the basal levels rather be low. On the other hand, the estimation of changes in estradiol by measurement of the production rate found that it were in low normal range and a sharp several-fold increase upon withdrawal with a subsequent fall to normal values after a period of abstinence (Hellman, Fukushima and Roffwang, 1975). Therfore, the measurement of the production rate of $E_2$ in male may be more precise diagnosis of narcotic effect

on estrogens. However, the reduction of $E_2$ concentrations could be observed in monkeys treated with 6.0 mg/kg/day during treatment and posttreatment periods and related to the lowered testosterone precursor.

There were a few documents of acute and chronic effects of morphine on TSH and $T_4$ levels. In human, opiates were either no effect (Tolis, Hickey and Guyda, 1975; Reid et al., 1981) or increase in TSH levels (Stubb et al., 1978; Delitala, Grossman and Besser, 1981; Devilla et al., 1985; Kuhn and Saltiel, 1986; Pende et al., 1986; Pende et al., 1987; Szekely et al., 1987). In this study, the acute effects of morphine in male cynomolgus monkeys were found the significant rise in serum TSH and $T_4$ levels in some points. Thus chronic effect of morphine at present could also detect only some changes in serum TSH and $T_4$ levels. Almost all adult monkeys showed a relatively consistent serum TSH levels while in pubertal monkeys the values during posttreatment period were increasing. This elevated TSH levels were compatible with the reduction in $T_4$ levels during the second half of posttreatment period (day-438 to day-466). This simultaneous changes raise from the reason that $T_4$ plays a major role in regulation of negative feed back mechanism to hypothalamic TRH and pituitary TSH secretion and release (Ingbar and Breverman,1986). In addition, adult monkeys recieved the same dose of morphine as in pubertal monkeys(3 mg/kg/day) also exhibited a similar reduction in $T_4$ levels at the same time. The blood collection during this time was done after the long elapsed time and the monkeys completely recovered. The $T_4$ reduction was notably coupled with the cortisol rise. It may suggest that during that time

monkeys may feel stress and unaccustom to the blood collection after being discharged for a long time. Stress may resulted in increase of cortisol levels (Mason, 1972) and decrease in TSH levels with consequent lowered $T_4$ levels (Christy, 1978). Surprisingly only the high dose of morphine (6.0 mg/kg/day) treated to adult male monkeys could exhibit effective stimulation of $T_4$ secretion. In contrast, the long-term administration of morphine in lower doses did not show any obvious changes in serum $T_4$ levels in adult male and female monkeys (Malaivijitnond, 1990). It may conclude that the influence of morphine on hypothalamic-pituitary-thyroidal axis in adult monkeys require relatively higher dose of morphine than the influence upon hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axis. In keeping with the present study, Azizi et al.(1972) observed that heroin and methadone addictions in human resulted in an increase in the thyroxine binding capacity of the serum thyroxin binding globulin(TBG). In contrast to the rat, morphine induced a reduction in TSH and $T_4$ levels (Morley et al., 1980; Gabriel, Simkins and Millard, 1985; Bhargave et al., 1989).

The present result has been conflicting to the other previous reports observed that PRL decrease during withdrawal period in human (Gold, Sweeney and Pottash, 1979) and rats (Mioduszewski, Zimmermann and Critchlow, 1982), since at here PRL levels are increased during posttreatment period. Several indirect lines of evidence available overwhelmingly suggested that the main effect of morphine and related narcotics is to inhibit dopaminergic activity in the hypothalamus (Van Vugt, Bruni and Meites, 1978; Gudelsky and Porter, 1979; Van Loon, De Souza and Shin, 1980; Delitala, Grossman and Besser, 1982;

Haskin et al., 1981). Blockade of dopaminergic activity would lead to an increase in serum PRL levels (Gold, Sweeney and Pottash, 1979; Van Vugt et al., 1979; Van Loon, De Souza and Shin1980; Delitala, Grossman and Besser, 1981). A consequence of continued treatment and the resulting opiate dependence is the abstinence syndrome subsequent to opiate antagonist exposure or the cessation of morphine treatment. Opiate withdrawal is accompanied with the dopaminergic hyperactivity (Lal, 1975), both dopamine(DA) and its major metabolite, dihydroxyphenylacetic acid(DOPAC), showed an increased concentration in the medial basal hypothalamus as well as the preoptic area-anterior hypothalamus regions (Gabriel, Simkins and Millard, 1985). Therefore, the serum PRL levels were significantly reduced during the peak of opiate withdrawal observed from the withdrawal signs and symptoms (Gold, Sweeney and Pottash, 1979; Mioduszewski, Zimmerman and Critchlow, 1982). In contrast, Gabriel, Simkins and Millard(1985) reported serum PRL levels increased 15 minutes into morphine withdrawal which related to the increment of beta-endorphin-like immunoreactivity (B-END-LI). It has been demonstrated that naloxone-induced withdrawal in morphine-tolerant rats induced an increase of plasma B-END-LI (Antonio Martinez et al., 1990; Vargas et al., 1992). It is tempting to speculate that PRL rise in the present result may come from an adaptation of monkeys to increase endogenous opiate secretion, particularly B-EP after a prolonged suppression by exogenous opiate (morphine hydrochloride). In sum, it resulted in increase basal PRL levels during posttreatment period.

Integral Alterations in Hormonal Levels during Chronic Morphine Treatment

It has been different from the study of chronic effect of morphine in rats (Cicero, Wilcox et al., 1976; Cicero, Badger et al., 1977; Cicer et al., 1989; MacDonald and Wilkinson, 1991) or narcotic addicted patients (Eisenmann, Fraser and Brooks, 1961; Cushman, 1973; Ho et al., 1977; Celani et al., 1984, Malik et al., 1992) in which plasma morphine levels were particularly maintained in the effective dose, and, then, the target endocrine glands were continually mediated by morphine. It would be a vexatious problem to inject many times a day of morphine to animal subjects in a long duration. Therefore, the objective in this study was focused on the effect of single daily injection of morphine on hormonal changes and the result from morphine accumulation in body fate after single daily dose for some duration prior to recieving the next dose. At present, the hormonal determination was done 20 hours after each daily subcutaneous injection of morphine hydrochloride. As referred to acute effect, all hormonal changes after subcutaneous morphine administration preferentially recovered to the pretreatment values within 10 hours. Therefore, it could not observe any obvious hormonal alterations in all monkeys during long-term treatment of morphine hydrochloride. It may suggest that the interval time between each dose injection is long enough for monkeys to adapt themself before recieving the next dose but it is not long enough for monkeys to display a severe withdrawal symptoms. After a few days of morphine cessation would be notably found many changes in hormonal levels into withdrawal peroid. From these reasons, the hormonal

alterations in each monkey group will be discussed separately.

1. Adult monkeys treated with 1.5 mg/kg/day morphine

Since all hormonal levels were rather consistent. It means that morphine in dose 1.5 mg/kg/day deprives of the effect on hormonal levels when the blood sample collected. The statistically alterations in hormonal levels could be found only in monkey no.93. Monkey no.93 was selected to participate in this study eventhough he was borned out of the colony, because the high PRL level was detected in the serum sample taken after a single injection of ketamine anaesthesia during a pilot study. After that, the monkey was tested the ketamine effect on PRL levels, then, he showed a rapid rise of PRL levels after ketamine injection. The PRL rise was decreased to the basal level at approximately 2.5 hours after ketamine injection (Malaivijitnond and Varavudhi, 1994), whereas a single morphine injection could sustain PRL levels until 4.5-10.5 hours. In order to assess that if ketamine hydrochloride could potentiate the effect of morphine on PRL secretion in the mechanism of milk production, the monkey was given morphine hydrochloride 1.5 mg/kg/day for 130 days and the sequential blood samples were taken after ketamine anaesthesia. It is of interest that the monkey could be extruded only watery fluid from the mammary glands in some duration. It may be resulted in the lowered $E_2$ levels during treatment period whereas testosterone levels were rather consistent. Since $E_2$ has an important role for the poliferation of mammary cells (Patton and Jensen,1976; Austin and Short, 1984). Serum PRL values were consistently decline during treatment period. Monkey no.93 may be in a mild withdrawal state from which a hyperactivity

of dopamine decreased PRL levels (Lal, 1975; Gold, Sweeney and Pottash, 1979; Garbiel, Simpkins and Millard, 1985) during the time of blood withdrawal. It may suggest that the effect of ketamine on PRL levles could not overcome the hyperactivity of dopamine from the effect of morphine withdrawal.

2. Adult and pubertal monkeys treated 3.0 mg/kg/day morphine

Morphine hydrochloride in dose 3.0 mg/kg/day could represent an effect on some hormonal levels in male cynomolgus monkeys. It is of interest that all monkeys exhibited lowered $T_4$ levels during the second half of posttreatment period (day-438 to day-466 in pubertal monkeys and day-457 to day-485 in adult monkeys). In contrast with the result in heroin and methadone addicts, they exhibited an increase in $T_4$ levels compared to normal subjects (Azizi et al., 1972; Ho et al., 1977). In addition, Azizi et al. (1972) found that thyroxin binding capacity of the serum thyroxin binding globulin (TBG) was increased concomitantly with a slightly decline in free thyroxin ($FT_4$) levels. That is, an increased extrathyroidal thyroxin pool and a decreased fractional and a normal absolute thyroxin turnover (Robbin and Nelson, 1957; Oppenheimer, 1968; Osathanondh, Tulchiwsky and Chopra, 1976). However, the lowered $T_4$ levels in adult monkeys showed a positive correlation with TSH levels whereas it was an inverse relationship in pubertal monkeys. Wolf et al.(1980) reported that TSH correlated inversely with thyroid function in primary thyroid disease states whereas in normal function these was a direct correlation between TSH levels and the functional response.

The cortisol rise during withdrawal period suggested that the monkey felt stress (Mason, 1972; Puri, Puri and Kumar, 1981; Fuller et al., 1984; Munck, Guyre and Holbrook, 1984). Since synthetic glucocorticoid dexamethasone inhibited testosterone production (Welsh, Bambino and Hsueh, 1982; Bradley, 1990; Lalau et al., 1990; Urban, Miller and Dorsa, 1991; Orr and Mann, 1992), then, the testosterone drop taken together with cortisol rise has been observed. In adult monkeys, the pituitary-gonadal function has been already in a steady state the morphine could decrease testosterone levels with gradually return to the normal levels thereafter. Whereas in pubertal monkeys when circulating testosterone oscillated, the morphine effect on testosterone levels could not be found (Steiner and Bremner, 1981; Meusy-Dessolle and Dang, 1985).

Adult monkey no.509 could be continued to extrude milky excretion from the mammary gland even during morphine cessation. This evidence was in agreement with the suggestion that continued elevation of PRL is not necessary for the maintenance for normal lactation (Quigley and Haney, 1980).

3. Adult monkeys treated with 6.0 mg/kg/day morphine

During early treatment peroid morphine produced transient decrease of testosterone, $E_2$ and cortisol levels with a rapid rise to normal levels thereafter. Generally, results are in agreement with a number of investigators that morphine suppresses testosterone, $E_2$ and cortisol levels in monkeys and human (Hellman, Fukushima and Roffwang, 1975; Tolis, Hickey and Guyda, 1975; Grossman et al., 1982; Taylor, Dluhy and William, 1983; Grossman et

al., 1986; Pende et al., 1987; Malaivijitnond and Varavudhi, 1993). It is tempting to speculate that monkeys adapted themself to morphine effect in dose 6.0 mg/kg/day on testosterone secretion earlier than in dose 3.0 mg/kg/day. Owing to lowered testosterone levels in monkeys treated with 3.0 mg/kg/day morphine were gradually increased to pretreatment values during morphine administration whereas monkeys treated with 6.0 mg/kg/day morphine showed a promptly recover.

Stress reduces testosterone (Fuller et al., 1984) and $E_2$ in rhesus monkeys (Channing et al., 1977) but increases cortisol levels in squirrel monkeys (Brawn, Schalch and Reichlin,1971) and human (Oltras, Mora and Vives, 1987). Following these, the lowered testosterone and $E_2$ levels with cortisol rise when morphine withdrawal should result in stress effect. Notably, morphine in dose 6.0 mg/kg/day showed a significantly elevation in $T_4$ levels during treatment period whereas lower doses (0.1-0.8 mg/kg/day) could not increase $T_4$ levels in female cynomolgus monkeys (Malaivijitnond, 1990). It means that the high dose of morphine is needed to modulate thyroid function, therefore, morphine effect on thyroid levels has been difficult to detect. Since thyroid hormone synthesis and secretion are regulated by both extrathyroidal (TSH) and intrathyroidal by means of autoregulatory mechanisms (Greenspan, 1991).

In sum, it may conclude that the hormonal alterations in any doses of morphine in male cynomolgus monkeys depend upon the threshold, sensitivity and idiosyncrasy (genetically determined

abnormal reactivity to chemical, Gilman et al., 1985) of each target organ in each monkey. Therefore, the hormonal changes after morphine administration could be considered so in susceptible monkeys.

Metabolic Turnover Rate of Morphine

Morphine pharmacokinetics have been assessed in recent studies using both radiolabeled morphine (Brunk and Delle, 1974; Olsen, 1974) and unlabeled morphine quantitated in blood by radioimmunoassay (Findlay et al., 1978; Yoburn et al., 1985; Sear et al., 1989) or high-liquid performance chromatography (HPLC) techniques (Rnne et al., 1984; Westerling, Frigen and Hoglund, 1993). Which analytical technique available for morphine measurement is particularly considered about the sensitivity and specificity of that technique. Radioimmunoassay techniques have done much to increase the specificity of analytical methods for distinguishing the measurement of unchanged morphine from its metabolites which depends on the antibodies used. Also, HPLC techniques are capable of differentiating between unchanged morphine and its metabolites (Greene and Hug, 1982). However, these both techniques have a relatively low order of sensitivity. Therefore, the study of morphine pharmacokinetics at present is concerned to use the radiolabeled morphine. Because the tritiated morphine was given intravenously only 20 uCI (1 mCi = 0.0038 mg), an approximately 1 percent of the total unlabeled drug (Curry and Whelpton, 1983). It was a small amount of morphine that should not interfere the drug disposition which daily injected by subcutaneous route to all monkeys. In addition, this technique is taken an advantage about the

validity of precision since it require fewer steps for measurement, only pipetting the plasma sample and adding the scintillation fluid. Unfortunately, use of radiolabeled morphine which is a sensitive technique but lacks the specificity required for detailed metabolism studies (Olsen, 1974). Since, without plasma extraction, the drug disposition determened both unchanged morphine and its metabolites simultaneously.

The biological plasma half-life ($t_{1/2B}$) of morphine obtained in the present work is 308.21 $\pm$ 20.30 minutes comparable to those reported in normal man (2.9 $\pm$ 0.5 hours; Stanski, Greenblatt and Lowenstein, 1978; 1.56 $\pm$ 0.61 hours; Westerling, Frigren and Hoglund, 1993), female rhesus monkey (102-202 minutes; Rane et al., 1984) and the rat (8.30 hours; Yoburn et al., 1985). However, the estimated half-life in this study is somewhat longer than the previous report in female cynomolgus monkeys (1 1/2 hours; Setheetham, Varavudhi and Yodyingyuad, 1991). Similarly, Rigg et al. (1978) found that the half-time of the elimination phase to be significantly shorter in female (110 minutes) than in male surgical patients (173 minutes) given morphine intramuscularly. The two-compartment model best explain the following data of which are derived from study in which morphine has been administered intravernously in order to emphasize distribution and elimination process while avoiding absorption factors. This is consistent with the previous report of Rane et al. (1984); Yoburn et al. (1985); Shelly, Quinn and Park (1989); Westering, Frigren and Hoglund (1993). In spite of morphine was given daily to the monkeys by subcutaneous route, morphine metabolism was determined after intravenous

administration. Since route of administration does not alter plasma half-life, while alter plasma levels of free morphine (Brunk and Delle, 1974; Iwamoto and Klaassen, 1977). Therefore, the half-life values obtained from intravernous administration may interchange to explian the daily subcutaneous injection of morphine disposition. Morphine is rapidly absorbed after intramuscular and subcutaneous injection, producing plasma levels of free morphine from 15 minutes to 3 hours, which are significantly higher than levels after intravernous administration (Brunk and Delle, 1974). Additionally, systemic availability of intramuscular morphine is completely absorbed (Stanski, Greenblatt and Loenstein, 1978). Morphine absorption of monkeys by the intramuscular route does not differ greatly from that found with subcutaneous administration, but the former route yield an earlier and greater peak (Way and Adler, 1961a). Therefore, morphine administration was more preferably serve by subcutaneous route at here.

It is likely that the biological half-lives in monkeys treated with 3.0 mg/kg/day morphine are lesser than the other monkeys. It may result in anaesthetic effect. Since the monkeys treated with 3.0 mg/kg/day morphine were anaesthetized with 30 mg ketamine initially and, thereafter, maintained with small doses (10-15 mg) given at 45 to 60 minutes intervals until 360 minutes (Rane et al., 1984). Whereas the remaining groups recieved ketamine 30 mg only during intravenous $^{3}H$ morphine administration. The effects of anaesthesia on drug metabolism are complex (Shelly et al., 1988), it may influence by three separate effects : on drug distribution, on hepatic blood flow and on drug elimination (Sear et al., 1989a).

General anaesthesia would, however, be expected to affect morphine pharmacokinetics insofar as anaesthesia is associated with changes in ventilation, pH, cardiac output and tissue perfusion (Greene and Hig, 1982). Sear et al.(1989) found that morphine elimination half-life in anaesthetized patients (153 minutes) was lesser than awake patients (207 minutes). Shelly et al.(1988) demonstrated that the pharmacokinetics of oral control release morphine was a statistically significant difference between the two groups of patients recieved two different anaesthetic techniques : the first group recieved general anaesthesia with neuromuscular blockade and the remainder recieved lumbar epidural analgesia with general anaesthesia but without neuromuscular blocking agents. This difference was because of the varying concentrations of morphine metabolites and may reflect the change in splanchnic blood flow produced by lumbar epidural analgesia. Hypotension is a well document side effect of epidural analgesia and the total hepatic blood flow decreases in proportion to the degree of hypotention (Greenway, 1983). It was assumed that liver is the predominant organ involved in the metabolism of morphine (Bodenham, Quinn and Park, 1989) and the other extrahepatic sites such as the gut, kidney and lung may be cooperated (Bable, 1982). It has been observed that stress reduces hepatic blood flow, thus, the hepatic extraction ratio with consequently the biotransformation of morphine are decreased (Shelly et al., 1988). Thus, the monkeys treated with 1.5 and 6.0 mg/kg/day morphine which were recieved only a single injection of ketamine when the pharmacokinetics studied may feel stress and have longer half-lives of unchanged morphine in the blood circulation. Since morphine metabolites, mainly in conjugated forms, were easily to excrete by kidney (Gilman et al., 1985), In

addition, the impurity of $^{3}H$-morphine stock solution which increased by days during using in these monkeys may interfere the disposition of morphine.

Chronic administration of morphine or any other chemical agents in large doses could easily result in the alteration of the animal ability to dispose of morphine or those chemical agents (Way and Adler, 1961). The turnover rate of morphine during treatment period became gradually decline which in turn the biological half-life was increase. A decreased urinary excretion of conjugated morphine could occur during chronic morphine adminstration (Way and Adler, 1961). In 1956, Axelrod found that in chronically morphinized male rats there was a marked reduction in the ability of the liver microsomes to demethylate morphine. Additionally, with chronic administration, mobilization of the biliary stores of free and bound morphine would augment the effect of injected morphine and lead to higher morphine levels with subsequently increase half-life (Way and Adler, 1961). Ryan, Parker and William(1972) suggested that plasma protein binding of morphine may be increased in addiction, therefore, the depot of morphine in blood circulation was also increased. Since the plasma protein binding of morphine was found to be 30 percent in female rhesus monkey (Rane et al., 1984). Practically, the increase in pH is accompanied by a consistent increase in morphine binding (Olsen, 1974). Inturrisi and Verebely (1972) found that the plasma half-life after a single dose of methadone was shorter (15 hours) than that in methadone maintenace patients (25 hours).

Thereafter, the turnover rate trended to increase to pretreatment values, even during treatment period, particularly in monkeys treated with 1.5 mg/kg/day morphine. It was seemingly to be a dispositional tolerance. Dispositional tolerance results from change in properties of the agent in the organism, such that reduced concentrations are present at the sites of drug action. The most common mechanism is an increase rate of metabolism (Gilman et al., 1985). In rats made gradually tolerant to 150 mg/kg morphine in approximately a month, the blood levels of free morphine were found to decrease more rapidly than they did in non-tolerant animals. The difference was attributed to impair conjugation of morphine in the non-tolerant animals resulting from inhibition of glycogen synthesis by injected morphine (Szerb and McCurdy, 1956). Additionally, the long-term administration of morphine has progressively disruptive effects on the mechanisms responsible for re-absorption of bound morphine from the gut (Way and Adler, 1961). Animal studies suggested that the biotransformation (Misra et al., 1973; Masten et al., 1974) and elimination rates (Verebly et al., 1975) of methadone increased in chronic oral treatment. The concentration of free and bound morphine at 90 minutes and 4 hours were found, in general, to be higher for most tissues in tolerant than in non-tolerant animals (Wood, 1954).

Surprisingly, the turnover rate in monkeys treated with 6.0 mg/kg/day was rather consistency throughout the study period. Baselt and Casarett(1972) reported that in methadone maintenance patients recovery of metabolite-1 (2 ethylidene-1,5-dimethyl-3,5-diphenyl pyrrolidine) and methadone from the urine increased from 20

to 90 percent of the daily dose as the dose of methadone increase from 30 to 160 mg/day. In addition, morphine being a histamine releaser causes self-depression of absorption after subcutaneous adminstration (Milthers and Schou, 1958). It is well known that opioid agonists (especially in a high dose range) could modify cardiovascular functions, suggesting that morphine may inhibit its own absortion and decreases its own volume of distribution (Miyamoto et al., 1993) as a result of its hypotensive effect (Greene and Hug, 1982). Therefore, the turnover rate levels during treatment period in this monkey group were not altered. Fry et al.(1929) represented that fraction eliminated bore no relation to hight, weight, volume of urine or length of addiction, but was directly proportion to the quantity administered.

The plasma morphine concentration could detect in the blood until 24-48 hours (Brunk and Delle, 1974; Shelly, Quinn and Park, 1989; Mitchell et al., 1991). A relatively small amount of morphine appeared in the feces (Fry et al., 1929). Since in the rhesus monkey, 79 to 85 percent of a given intravenous dose of morphine was retrieved in the urine within 6 hours as morphine -3-glucuronide (Rane et al., 1984). The prolonged presence of morphine in blood during the first six hours may represent its continued metabolism, release of drug as well as its metabolites from tissues, enterohepatic circulation, persistence of a metabolite, or a various combination of these. The small amounts of morphine detected by radioimmunoassay 48 hours after intravenous addministration of the drug may be albumin bound (Spector and Vesell, 1971). Therefore, the blood sample collected at here did lastly at 6 hours. However, the

variation in turnover rate from animal to animal within each group were great during the onset of study. Szerb and McCurdy(1956) demonstrated that the variation and the lag were to be expected since the appearenace of conjugated morphine was dependent upon conjugation processes, which in turn were subject to influence by dosage, physiological state of the animals, prolonged morphine administration etc. In accordance with its kinetics, morphine disposition are independent of the dose, whereas the absolute concentrations achieved in plasma and brain are proportional to the dose (Greene and Hug, 1982). Therefore, the intensity and duration of morphine effects are proportional to the dose.

Testicular Measurement in Relation With Its Function

Result from figure 50-53 showed that morphine was no significant effect on testosterone levels in all male monkeys, however the levels during early withdrawal period in adult male monkeys treated with 3.0 and 6.0 mg/kg/day exhibited a maked decline. Indeed, acute and chronic morphine administration significantly depressed serum testosteronse levels by an action on hypothalamic-pituitary-gonadal axis in the rat (Cicero, Meyer et al., 1976; Cicero, Badger et al., 1977; Cicero, Bell et al., 1977) and rhesus monkeys (Gilbeau et al., 1984). The lowered testosterone levels were returned to control levels after a single morphine injection for 7 hours in the rats (Cicero, Wilcox et al., 1976) and more than 10 hours in these male cynomolgus monkeys (as referred to acute effect study). Therefore, it could not be observed any alteration in serum testosterone levels which taken 20 hours after each morphine

injection. During precipitated morphine withdrawal, noradrenergic neurons in the locus coeruleus of the rat increased their activity (Crawley et al., 1979; Gabriel, Simpkins and Millard, 1985). The brain noradrenergic system is stimulatory to LH secretion (Gabriel, Simpkins and Millard, 1985). Therefore, the initial increase in norepinephrine turnover should associate with a marked increase in LH and testosterone levels during withdrawal period.

Morphine produced the loss of weight in adult male cynomolgus monkeys in a dose-dependent manner. The higher treatment displayed the more and longer time in reduction of body weight. The highest dose of morphine treatment (6.0 mg/kg/day) showed a progressive decline in body weight throughout treatment period. This lowered body weight could be explained in part, at least, on the basis of a reduce intake of food and water during the treatment period (Rennels, 1961), since morphine reduced a metabolic rate (Adriani, 1970). Morphine allays a hunger by decreasing the motility and increasing the emptying time of the stomach (Wood-Smith and Stewart, 1964; Adriani, 1970; Wood and Wood, 1982). Light(1930) studied a series of morphine addicts before and after treatment with scopolamine which resulted in the positive change of loss of weight. Rennels(1961) reported that morphine treatment (6.0mg/kg/day) in rat age 47 days for 14 days significantly reduced the body weight. However, the reduction in weight gain induced by the overcrowding of rats could reverse by daily administration of naltrexone (Amir, Galina and Amit, 1979). It is indicative that endogenous opioids may under such chronic conditions act, probably indirectly, as a suppressor of food intake. Moore(1965) found that malnutrition, induced by protein

deficiency, depressed both growth rate and reproduction in the rats. Of interest, all of adult male monkeys showed a marked decline in body weight during early withdrawal period, particularly in the highest dose treatment (6.0 mg/kg/day) and following with a gradually increase to a basal lavels, thereafter. Mellet and Wood (1956) subcutaneously injected 30 mg/kg/day morphine sulfate to adult female rhesus monkeys, after about 2 months the animals demonstrated about 6 to 8 hours of withdrawal symptoms during each 24 hours with a progressively weight loss. This is consistent with other report that morphine implanted rats were significantly lighter than controls by 12 hours after pellet removal with a nadir at 48 hours, thereafter, the body weight was slightly increased but remained significantly lighter untill the end of study period (120 hours)(Yoburn et al., 1985). Usually, a considerable loss of weight is found during the first 48 hours of withdrawal period concomitantly with many other associated withdrawal symptoms such as pupil dilation, insomnia, nausea, vomitting and diarrhea in addicted patients. These signs and symptoms reach their height by the end of about 72 hours (Wood and Wood, 1982). Ling, Tappe and Inturrisi(1984) concluded that the loss of weight during withdrawal period was due to the loss of body fluids by urination, diarrhea and salivation. After successful withdrawal when the drug has been withheld for from 10-14 days the addicts were taking food normally, has regained their weights and could present the picture of well being (Light, 1929)

In this study, the loss of body weight was accompanied with reduction of the testicular size, but did not correlate with the serum testosterone changes. This results were different from the rat

since subcutaneous implantation with 75 mg of morphine during a 3-day period did not decrease significantly in testicular weight. Whereas serum testosterone levels was reduced within 6 hours and the weight of the seminal vesicles and prostates were later reduced by 24 hours (Cicero, Meyer et al., 1976). In addition, the male rats represented these evidents, body weight and food and water intake were no differences when compared with placebo-implanted rats (Cicero et al., 1975). Barraclough and Sawyer(1955) showed that the effect of morphine on the ovarian cycle did not come from a result of under nutrition. Therefore, the reduction of the testicular size herein may result in the direct effect of morphine on the testicular tissues. Since, quantitative reductions in spermatogenic cell population with all stages of spermatogenesis were found in testicular sections among rats treated with morphine 50 mg/kg/day for up to 9 weeks which were reversed within 13 weeks of drug withdrawal (James, Heywood and Crook, 1980). B-EP, ACTH and other peptide derived from POMC have been demonstrated in relatively high concentration in human semen (Sharp and Pekary, 1981) and in reproductive organs of rodent (Tsong et al.,1982; Tsong, David et al., 1982; Chen et al., 1984; Lundblad and Roberts, 1988). Intratesticular injection of B-EP significantly decreased testosterone response to intraperitoneal LH treatment (Chandrashekar and Bartke, 1992). Also, B-EP decreased both basal and hCG stimulated testosterone release from interstitial cells culture (Kant and Saxena, 1993). On the other hand, intratesticular administration of naloxone caused a significant increase in testosterone levels (Kant and Saxena, 1993) and stimulation of Sertoli cell proliferation and secretion (Gerendi,1991). This may

suggest that there are opiate receptors at Sertoli and Leydig cells. Nevertheless, there was no published report about opiate receptors in these cells, only the presence of opiate receptors in the vas deferences (Hughes, Kosterlitz and Leslie, 1975). In sum, the testicular size reduction in adult male cynomolgus monkeys after long-term treatment with morphine hydrochloride may result from the reversible direct effect of morphine on testicular tissue and spermatogenesis. These deviations could recover at least 1 month after the drug withdrawal since monkey no.512 and 509 could masturbate themself and produced many motile sperm during that time.

Testicular size and testosterone levels proved to be a highly reliable index of maturation (Glick, 1979). The pubertal males in this study has an average age at 4.46 $\pm$ 0.65 years. The pubertal stage as indicated by the descent of testes into scrotal sac (Sade, 1964), the increasing in serum testosterone concentrations accompanied with the onset of spermatogenesis as well as canalization of the seminiferous cords in the testis (Steiner and Bremner, 1981; Dang and Meusy-Dessolle, 1985). The mean age at the begining of the testosterone rise in captive cynomolgus macaque was 43.1 months (Meusy-Dessolle and Dang, 1985). Testosterone concentration in the study (7.69 $\pm$ 1.69 ng/ml) was similar to Meusy-Dessole and Dang (1985) measurement (approximately 7 ng/ml). As the animals achieved puberty, the testosterone level continued to increase up to adult stage at about 5.6 years of age. Because the pubertal monkeys displayed a maturational increase in testicular size including weight gain during entire treatment period. It may speculate that morphine at the dose of 3.0 mg/kg/day over a period of 74 days is

insufficient to suppress major physical and hormonal changes of pubertal monkeys completely, although withdrawal of morphine treatment were followed by a more pronounced increment of testicular size. There were many reports in some macaques including the Japanese macaque (Imanishi, 1960), bonnet macaque (Valerio, Pallotta and Courtney, 1967; Glick, 1979) and rhesus macaque (Sade, 1964; Valerio, Pallotta and Courtney, 1967) which exhibited a mating period and the effects of season were superimposed on these growths (Glick, 1979). But the captive male cynomolgus monkey has no breeding season (Valerio, Pallotta and Courtney, 1967; Varavudhi, Tangpraputtigul and Asawaroengchai, 1982). Therefore, any morphological and hormonal changes during morphine treatment in these cynomolgus monkeys would definitely due to the effect of morphine rather than changes in seasonality.

Galactorrhea Symptom

In the rat, suckling evokes a rise of plasma levels of PRL, GH,cortisol, while FSH and LH levels decline (Chiocchio et al., 1979; Selmanoff and Wise, 1981; Riskind, Millard and Martin, 1984; Selmanoff and Gregerson, 1986). This pattern of hormonal responses is remarkedly similar to that elicited by opiate peptides; that is, opiates release PRL, GH and ACTH and inhibit the release of gonadotropins (Pang, Zimmerman and Sawyer, 1977; Spampinato et al., 1979; Buckingham, 1982; Spiegel, Kourides and Pasternak, 1982). The similarlity of the opiate-induced and suckling-induced responses suggest that endogenous opiate may participate in milk production (Selmanoff and Gregerson, 1986). Since suckling is a major factor

responsible for maintaining optimal secretion of milk in puerperal woman (Quigley and Haney, 1980). Indeed, mammary growth is largely controlled by hormones arising from the anterior pituitary gland, from the ovaries, and from the adrenal cortex. During pregnancy the placenta is an additional source of both steroid and polypeptide hormones. In rodents, for duct growth, $E_2$, GH and adrenal steroid are necessary; lobulo-alveolar growth will ensure if prolactin and progesterone are also added to this triad and lastly, prolactin and adrenal steroids are main known factors required for regulating milk secretion (Austin and Short, 1984; Silberstein et al., 1994). At cellular levels, PRL appears to act on the plasma membrane and presumably induces changes in the nucleus through a second messenger (cAMP) mechanism. Together with insulin, PRL induces new mRNA and a number of the enzymes responsible for the synthesis of milk components. This hormone also regulates serum lipid uptake by the lactating mammary gland. Two proteins, UDP-galactosyl transferase and alpha-lactalbumin, constitute the enzyme that synthsizes lactose, so-called lactose synthetase are also under the regulation of PRL. (Turkington and Hill, 1969; Patton and Jansen, 1976). Since alpha-lactalbumin is one of the principal (whey) protein of milk, the synthesis of milk protein is directly related to synthesis of lactose (Patton and Jansen, 1976). PRL could induced an up-regulation of its receptors, since PRL increases receptor levels in cell culture and bromocriptine, a PRL inhibitor, causes a decrease in both PRL and its receptors (Greenspan, 1991).

The mammary glands are present in both sexes although generally poorly developed in the male, and are normally functional

only in the female (Austin and Short, 1984). Therefore, galactorrhea in the male is a relatively rare disorder, generally occurring with hypogonadism and hyperprolactinemia (Wieland et al., 1967). This different in sexual dimorphism would lead to produce and excrete less amount of milk found in these experimental male monkeys. As previously referred, PRL is the most important hormone in the initiation of milk production, and high doses of female sex steroids, $E_2$ and progesterone, inhibited the PRL activity at the mammary gland (Turkington and Hill, 1969). Moreover, testosterone also found to inhibit normal differentiation of the breast tissue (Archer, 1980). Therefore, the initiation of lactation could be predicted on the sudden drop of high concentrations of $E_2$ and progesterone accompanied with an enhancement of PRL level (Tyson et al., 1975; Johnson and Everritt, 1984). In keeping with the present result, milk secretion was initiated by prolonged exposure with high PRL levels after morphine administration untill coupled with a sudden drop of testosterone level. Additionally, alteration of the other hormones, e.g. cortisol, in male cynomolgus monkeys as confirmed by acute effect study may be cooperated. However,the establishment of normal milk secretion may require the presence of several more hormones which were not determined in this study (i.e. insulin, GH, etc.) (Tyson et al., 1972). This study definitely showed that continued elevation of PRL is not necessary for the maintenance of normal lactation. If the other hormonal parameters are not appropiates a patient with hyperprolactinemia may not have galactorrhea, and a patient with galactorrhea need not have hyperprolactinemia (Quigley and Haney, 1980). This is in turn a stimulating excessive PRL secretion by morphine leading to

galactorrhea in only susceptible monkeys.

It may similarly to TSH hormone that the peak serum TSH after TRH was depended upon the basal TSH level (Sawin and Hershman, 1976). Since the monkey, for instance no.509, obligated with high basal PRL level showed the high peak of PRL after morphine and ketamine administration, and sustained this high level for a long time. PRL normal range for men is 0-390 mIU/L (Conrad, Breen and Leone, 1990).

Milk ejection could be observed only after pressure explusion on nipples of male monkeys at the present as in agreement with the recent reports in adult female cynomolgus monkeys (Setheetham and Varavudhi, 1993) and aged female cynomolgus monkeys (Intavat, unpublished data). Since morphine suppresses oxytocin secretion which stimulates the contraction of the myoepithelail cells which surrounds the alveoli, to induce expulsion of milk into the duct (milk let-down), with a consequent buid up of intramammary pressure which may cause milk to spurt from the nipple (Almeida and Pfeiffer, 1991). It is of interest that even cessation of morphine treatment, milk secretion could still occur. This may happen as long as 12 months in some monkeys after the drug withdrawal. As lactation progresses, the amount of PRL released at each suckling bout may decline, but the basal plasma concentrations often remain elevated, although how this is achieved is uncertain (Johnson and Everritt, 1984). Therefore, milk secretion in some monkeys could be observed in only some durations after daily single morphine injection which produced a transient rise of PRL and could not maintain the high

basal PRL levels prior to recieving the next dose of morphine injection. Since, galactopoiesis or the process of continued milk secretion is also dependent upon the function and integration of several hormones. Evidence from GH-deficient dwarfs and hypothyroid patients suggests that GH and thyroid hormone are not required for galactopoiesis (Greenspan,1991). The present study could not resolve the issue of how galactorrhea represents or terminates. However, it may postulate that the high levels of PRL compatible with the low levels of testosterone is a prerequisite for initiation of milk secretion in male cynomolgus monkeys.

Stress and Hormonal Alterations

Many investigators studied a variety of stress effect on ACTH/cortisol (Brawn, Schalch and Reichlin, 1971; Mason, 1972; Elvidge et al., 1976; Puri, Puri and Anand Kumar, 1981; Fuller et al., 1984; Oltras, Mora and Vives, 1987), PRL (Benker et al., 1990), GH (Brown, Schalch and Reichlin, 1971), FSH and LH (Puri, Puri and Anand Kumar, 1981; Fuller et al., 1984) and TSH and thyroid hormones (Christy, 1978; Greenspan, 1991) in monkeys and human. The pituitary-adrenal-cortical activity was profoundly used to indicate stress situation (Mason, 1972; Puri, Puri and Anand Kumar,1981; Fuller et al., 1984). Actually stress causes the release of immunoreactive CRF (ir- CRF) in several brain areas (Chappell et al., 1986). In addition, B-EP containing cells appear to remain under tonic CRF control since the CRF receptor antagonist alpha-helical $CRF_{9-41}$ inhibits B-EP release *in vitro* (Nikolarakis, Almeids and Herz, 1986). Consequently, B-EP and ACTH are concomitantly secreted by

pituitary gland in response to acute-stress or long-term adrenalectomy as well as *in vitro* in response to purified CRF and other secretagogues (Guillemin et al., 1977; Oltras, Mora and Vives, 1987). Considering the role of CRF in mediating various stress responses, the interaction between CRF- and POMC-containing neurons within the hypothalamus strongly suggests the involvement of hypothalamic endogenous opioidergic neurons in the regulation of the stress response (Trisdikoon, 1983; Sirinathsighji and Heavens, 1989; Przewlocki, Przewlocka and Lason, 1991).

Since endogenous and exogenous opiates could stimulate an increase in PRL levels and suppress testosterone, cortisol and $E_2$ levels with uncertain effect on $T_4$ and TSH levels in human (Hellman, Fukushima and Roffwang, 1975; Tolis, Hickey and Guyda, 1975; Jammes, Heywood and Crook, 1980; Reid et al., 1981; Taylor et al., 1983; Pende et al., 1987; Van Vugt, Webb and Reid, 1989; Malik et al., 1992). As considered to the acute and chronic effect of morphine in present study, the hormonal patterns in monkey no.525 treated with saline once a day was resemble in morphine treated monkeys. PRL levels in this monkey showed a marked progressive increase but testosterone and $E_2$ levels were gradually decline in successive blood samples taken after a single dose of the anaesthetic ketamine hydrochloride. Serum $T_4$ and TSH levels did not show any significant alterations as compared between blood samples obtained at different intervals. Cortisol levels trended to be high in monkey no.525 whereas it was reduced in monkeys treated with morphine. Since morphine evidently acts at hypothalamic-pituitary level to inhibit ACTH release (Volavka et al., 1979) while

stress acting through CRF and ACTH which stimulate rise of glucocorticoid levels (Munck, Guyre and Holbrook, 1984). On the other hand, recent clinical study showed that stress could affect TSH either at various levels of hypothalamic-pituitary-thyroid axis or by alteration of peripheral thyroid metabolism (Greenspan, 1991).

Galactorrhea seems to be a normal symptom in stress subject (Brambilla, Epstein and Kupperman, 1961). Similar situation may also operate in the male cynomolgus monkeys and other mammals as well. In estrogen primed rats which was treated by various stressful stimuli such as injection of 10% formaldehyde, severe cold (0 $^{o}$C), intense light and heat (35 $^{o}$C), restraint and starvation would result in mammary secretion and may accompanied with a significant decrease in thymus weight and increase in adrenals weight (Meites, Nicoll and Talwalker, 1963). In woman, many types of stressful stimuli, including lung infection, severe burns, stravation and harsh trauma, have also been observed to induce mammary growth or lactation (Brambilla, Epstein and Kupperman, 1961). However, milk secretion in monkey no.525 was not continuous. This may confirm previous studies that lactogenesis and galactopoiesis were caused from an orchestra of hormonal effect. It happens only in the appropiate ratio of hormonal parameters (Volpe' et al., 1972; Tyson et al., 1975; Austin and Smith, 1984). Bearing in mind that ketamine anaesthesia produced no significant changes in plasma cortisol, testosterone and gonadotropins levels but showed a progressive rise in PRL levels (Castro et al., 1981; Puri, Puri and Anand Kumar, 1981; Fuller et al., 1984). Therefore, monkey no.525 may sensitive to stress from cage restraint and/or anaesthetic administration than

other treated monkeys.

Summary

1. Long-term effects of morphine hydrochloride upon pharmacokinetics and hormonal changes were investigated in pubertal and adult male cynomolgus monkeys.
2. Morphine hydrochloride significantly modulated serum PRL, testosterone and cortisol levels in male monkeys, whereas TSH and $T_4$ levels were seemingly to unaffect after following for 10 hours. Apparently, the hormonal alterations could be able to recover within 10 hours after morphine injection.
3. Long-term study of daily morphine treatment on hormonal levels could not be able to detect any prominent alterations when the blood samples were taken 20 hours after each injection.
4. Monkeys could adjust themself after daily morphine administration and developed dispcsitional and pharmacodynamic tolerances.
5. The effect of morphine on hormonal levels in pubertal and adult male cynomolgus monkeys displayed a difference in time-course and latency.
6. Prolactin is the first and best marker to study morphine effect on hormonal levels.
7. Morphine could also induce watery or milky excretion after nipple expulsion in male cynomolgus monkeys particularly in susceptible monkeys by induction daily rise of PRL simultaneously with a sudden decline of a basal serum of testosterone levels.
8. Stress induced the onset of hormonal changes as in the response to morphine treatment, however, serum cortisol levels were increase during stress but decrease after morphine injection.

9. Cynomolgus monkeys exhibited the evidence of hormonal changes after morphine treatment and stress similar to human but conflicting with the rodent.
10. The cynomolgus monkeys should be an ideal non-human primate animal model for study morphine effect and transfering the result to human in which the study of narcotic effect has been concreted with moral and ethical standards.