Metabolic Turnover

The present result showed that the elimination patterns of morphine in these female cynomolgus monkeys were biexponential similar to previous reports in rhesus monkeys(Rane et al.,1984) and human subjects(Sawe et al.,1981). During pre-treatment cycle, the metabolic turnover (β) and half-lives($t_{1/2\beta}$) measured during follicular phase (D10-D12) and luteal phase (D22-D25) of 17 selected adult female monkeys with cycle length ranging from 28 to 39 days showed no statistical difference (β =0.38 and 0.32 % min^{-1}, $t_{1/2\beta}$= 183 and 216 min respectively). The values measured were within the range of adult female rhesus monkeys, older than 10 years, β = 0.34-0.68 % min^{-1} and $t_{1/2\beta}$= 102-202 min (Rane et al.,1984).

These data indicated similarity of metabolic turnover among closely related macaque species and values during active reproductive states have smaller fluctuation than aged momkeys with unknown record of fertility. Similar values with larger fluctuation is also found in healthy adult males of 26-32 years old, β = 0.23-0.65 %min^{-1} and $t_{1/2\beta}$ = 106-302 min. (Stanski, Greenblatt and Lowenstein,1978). However, more recent report showed extremely lower half-life with higher metabolic turnover of morphine in plasma of normal human subjects of both sexes with tendency of lower half-life in aged group than in adult group (Owen et al.,1983).

Turnover rate of morphine in female monkeys treated with 0.1-0.8 mg/kg/day morphine measured on day 30 and day 60 fluctuated within the same range of pre-treatment period except one monkey treated with 0.8 mg/kg/day morphine showed unusually high value measured on day 30 of treatment only. It remanined to determine whether this result was due to technical errors or to physiological adaptive mechanisms of individual animal in response to long term exposure of moderate dose of morphine.

High doses of morphine treatment showed greater fluctuation of turnover rate. In 1.6 mg/kg/day treated group the value tended to decline gradually and reach steady state around day 80 of the treatment, whereas 3.2 mg/kg/day of morphine treatment induced faster decline and reached the steady state approximately within 17-45 days of treatment which may imply as a state of physical dependence, physiological and behavioural disturbances(Verebely et al.,1975). This reduction of the turnover patterns may result from change in the pharmacokinetic properties and decrease in sensitivity of morphine in target cells (Jaffe and Martin,1985). It is noteworthy to stress that individual monkey in group of 1.6 mg/kg/day morphine treatment showed variable pattern of menstrual bleeding during entire period of treatment. Monkey no.92 showed regularity of the cycle throughout 143 days of treatment ; monkey no.93 postponed the cycle to 72 days and readjusted to 51 days on the second cycle; monkey no.95 returned to one cycle on days 113 of treatment only. However, all monkeys in group of 3.2 mg/kg/day morphine treatment exhibited anemorrhea throughout 120 days of treatment.

Withdrawal of morphine showed normal values of metabolic turnover measured on day 30 in all monkeys treated with 0.1-0.8 mg/kg/day morphine. In 1.6 and 3.2 mg/kg/day treated groups showed rebound effect followed by fluctuations and tends to approach normal pre-treatment values within 1-6 weeks. Only monkey no.95 and no.60 of 1.6 and 3.2 mg/kg/day morphine treated groups respectively, showed gradual increment of the turnover rate. Other monkeys showed immediate increase of turnover rates and required only a few days to return to the pre-treatment situation. These wide variations of individual recovery patterns may related with physiologically adaptive mechanisms, associated with mental disturbance which may refer to the levels of morphine dependence and addiction. Law et al.(1985) suggested that multiple adaptation processes occurred during chronic morphine treatment that is receptor desensitization, receptor down-regulation and increase in adenylate cyclase activity. This informations may be useful for further opiate addicted therapy particularly the duration of opiate detoxification stage.

E_2 and P Levels

The last 2 pre-treatment menstrual cycle length of 17 selected adult female cynomolgus monkey were between 28-39 days. The records were in comparable ranges reported by Shaikh, Naqvi and Shaikh(1978) 22-37 days; Dukelow, Grauwiler and Bruggemann (1979) 26-36 days; Varavudhi et al.,(1982) 27-35 days; Zumpe and Michael (1983) 27-40 days. Duration of menstrual flow 2-5 days was also similar to the range of 1-3 days previously reported in this monkey by Mahoney (1970), but similar to report of 1-5 days

by Yoshida(1986), and of 3-5 days in the rhesus monkeys (Johansson, Neill and Knobil,1968, Shaikh, Naqvi and Shaikh, 1978). In order to correlate patterns of serum E_2 and P levels for different phases of the menstrual cycle more accuracy than previously recorded, the cycles were further divided into 3 subgroups : i) 28-31 days, ii) 32-35 days and iii) 36-39, days respectively. Fluctuations of serum E_2 levels were found in all 34 pre-treatment cycles with E_2 peaks during D12-D16, D16-D21 and D19-D21 in subgroup i), ii) and iii), respectively. Serum P levels increased during D12-D26, D16-D31 and D19-D33 in subgroup i), ii) and iii), respectively. These results clearly indicated that all pre-treatment cycles are fertile cycles with normal ovulation occurred around D13-D20 and corpus luteum life lasted approximately 14-15 days in all cycle recorded.

During 100-143 days of morphine treatment, the menstrual cycles were lengthened in monkeys treated with higher doses of morphine.Usually monkeys treated with 0.4 and 0.8 mg/kg/day morphine could be able to readjust to their normal usual cycle during late treatment period. Similar patterns of the cycle during late treatment were also found in monkey no.92 treated with 1.6 mg/kg/day morphine. However, monkeys treated with 3.2 mg/kg/day morphine became amenorrhic and failed to have the cycle throughout treatment period. Incidentally, all monkeys that could readjust to normal cycle during morphine treatment always exhibited normal fluctuations of E_2 and P levels but the monkeys that failed to readjust to their usual cycle during treatment did not exhibit normal rise of serum E_2 levels showed total suppression of P levels. This suggested that

positive feedback of E_2 levels during morphine treatment period were not operated and ovulation with subsequent rise in P levels were prohibited(Norman et al.,1984, Orstead, Hess and Spies,1987). Our findings here are similar to the previous showed that finding of amenorrhea or irregular menstruation in heroin addiction (Gaulden et al.,1964) associated with absence cyclic gonadotrophin release, prolonged follicular phase and absence of E_2 positive feedback (Santen et al.,1975).

It is of interest to note that monkey no. 92 was the only animal treated with 1.6 mg/kg/day that still able to maintain her regular menstrual cycle throughout treatment period but with prolonged bleeding duration (menorrhagia) lasting as long as 8-9 days. The data suggested that disorder of prolong endometrium bleeding and the levels of ovarian steriods in morphine treatment are interdependent. It is difficult to explain effect of morphine on prolong bleeding , but altered bleeding duration may reflect to imbalance secretion of local intra-uterine and intra-ovarian agents responsible for luteolysis, presumably prostaglandins and/or oxytocin(Johnson and Everitt, 1988). Two other monkeys from the same treatment group were unable to maintain their normal cycle. The cycles of these monkeys were as long as 72-113 days with normal bleeding duration, whereas 3 other monkeys treated with 3.2 mg/kg/day morphine throughout 120 days failed to exhibit menstrual cycle. All of these monkeys, in addition, showed spontaneous galactorrhea.

Sexual Behaviours

The opiate appeared to interact in complex ways with endocrinology in production of behavioural effect. Unfortunately, there were no available reports of long term treatment of morphine on reproductive behaviours in higher non-human primates in the literature. The behaviours of the female monkeys during 0.1-3.2 mg/kg/day morphine treatment in this study are dose dependent. Ingeneral, approached, presented, invited to groom and grooming behaviours of the treated monkeys declined greatly in high dose groups. These behaviours remained low even during the first few cycle after morphine withadrawal.

Although it is generally accepted that E_2 is the major ovarian steriod responsible for restoration of normal sexual behaviours in ovariectomized monkeys (Michael, Herbert and Welegalla,1967, Michael and Zumpe,1984). Results of long term treatment of morphine in this study clarly indicated that this drug seriously affected sexual behaviours of the monkey independent of endogenous E_2 levels.

PRL Levels

Serum PRL ranges between 112 - 636 mIU/L during pre-treatment cycle . No difference was obseved statistically during all different parts ofthe cycle, and the values measured in this study were similar to these found in captive cynomolgus monkeys during the last 2 cycles prior to exhibit sucessful pregnancy after mating with fertile males: 400 - 1100 mIU/L (Varavudhi et

al.,1982) and other normal monkeys from the same colony; 140-640 mIU/L (Suwanprasert,1991). However,cynomolgus monkeys may secrete relatively lower serum PRL during normal ovulating cycle to the values as low as 109-244 mIU/L (Tangpraprutigul et al. ,1987). Similar situation were also reported by Varavudhi et al. (1992) in free-ranging monkeys obtained in certain regions of Thailand . The values measured in adult female rhesus monkeys was slightly low as 102-180 mIU/L (Wardlaw et al.,1980, Gindoff et al.,1988) but higher in chimpanzee: 569-793 mIU/L (Gosselin et al.,1983). Indeed, serum PRL levels of cynomolgus monkeys were very close to the value measured in adult fertile women:115-462 mIU/L(Tolis, Hickey and Guyda,1975, Petraglia et al.,1987), but post-menopausal women of 50 years olds exhibited slightly lower serum PRL levels: 211-225 mIU/L (Dawood, Khan-Dawood and Ramos,1986).

There were a few reports on measurement of serum PRL levels in female non-human primates during various phases of the menstrual cycle. However, the present result confirmed previous study in human females by Snowden et al.(1986) who found no significant alteration of PRL levels during follicular and luteal phases of the cycle.

Long term morphine treatment showed consistent rise in serum PRL levels. The response was dose dependent and significant rise of serum PRL levels were evidenced during the entire period of high doses treatment. Unfortunately, there was no other report of chronic morphine treatment on serum PRL

levels in primates to compare. However, acute intravenous injection of morphine induced sudden rise of serum PRL levels within a few minutes in rats (Pfeiffer et al.,1987), stump-tailed monkeys (Gold, Redmond and Donabedian,1979) and women (Tolis, Hickey and Guyda,1975). Shin et al., (1988) suggested that efficacy of morphine may be reduced after a prolonged release, owing to the depletion of the "releasable pool" in pituitary lactotroph. However, this study measured serum PRL levels, every 5-7 days intervals during morphine treatment. Oscillation of this hormone was evidenced with significant increase the serum level values over a prolong period of treatment (120-143 days). The situation seems to be similar with pituitary stalk section or hypophysectomy and autotransplantation in kidney capsule in rodents (Everatt,1954, Nikitovitch - Winer and Everett, 1959).

Hyperprolactinemia associated with spontaneous galactorrhea were found in female monkeys treated with high doses of morphine (1.6 and 3.2 mg/kg/day). It is of interest that only one monkey treated with 1.6 mg/kg/day morphine (no.92) exhibited regular cycle during entire period of treatment and failed to develop spontaneous galactorrhea inspite of the elevated serum levels of PRL identical to amenorrhic and galactorrhic monkeys in the same treatment group. The possibility of higher secretion of E_2 and P in this monkey may prevent establishment of galactorrhea in hyperprolactinemia condition, similar to the situation found during pregnancy in several groups of mammals (Varavudhi et al., 1982, Siriprasomsub,

1984) including higher primates and women (Johnson and Everitt,1988, Guyton, 1991).

As far as the acute effect of morphine on PRL secretion is concerned, this opiate may exert a direct action on its specific receptors within arcuate nucleus to suppress dopamine release from TIDA nerve terminals, thereby enhancing pituitary gland secretion of PRL (Groppetti et. al.,1977, Haskins et al. ,1981, Dawood, Khan-Dawood and Romos,1986) and may simultaneously inhibit neuromodulator of GnRH release (Grossman,1981, Petraglia et al.,1986). Indeed monkeys treated with high dose of morphine always have high serum PRL levels coincidenced with lower levels of sex steroids are comparable with clinically hyperprolactinemic women with high endogenous opiates in systemic circulation associate with amenorrhea (Petralia et al.,1987). It worth mentioning that a single systemic injection of dopamine agonist "ergocornine" into hypophysectomized and autotransplanted to kidney capsule of pregnant rats is sufficient to abolish autonomous secretion of PRL needed for maintainence of corpus luteum function(Varavudhi, Lobel and Shelesnyak,1966). Certainly, the rat in this situation would not be able to have oestrous cycle again unless the transplanted pituitary was retransplanted back into the median eminence(Everett,1956, Nikitovitch-Winer and Everett,1959). On the other hand, Shin et al.(1988) demonstrated that morphine still capable of stimulating PRL release in rat previously treated with a specific dopaminergic blocking agent, pimozide. He suggested that morphine may act partly through stimulation of

prolactin releasing factor (PRF) in addition to the effect on TIDA neurons. Recent evidence indicated that VIP is the potential agent responsible for direct stimutation of pituitary PRL secretion which may plays a physiological role as a PRF. This factor is detected in suprachiasmatic and paraventricular nuclei of rat, in human eminence, anterior pituitary and hypophyseal portal blood. Moreover, VIP is capable of stimulating PRL secretion at concentrations attainable in the hypophyseal portal blood (Kato et al.,1978, Clemens, Shaar and Smalsting,1980, Lam,1989). It remains to be determined whether i) opiate receptors existed in VIP producing cells, ii) morphine and other related opiates are capable of stimulating VIP synthesis and release, and iii) lactotroph also secrete VIP in addition to PRL.

Withdrawal of morphine showed immediate decline in serum PRL levels. Data favor the possibility of more direct effect of morphine on pituitary lactotroph than any other factors ever known. Sudden decrease of serum PRL levels after morphine withdrawal support the hypothesis of dopamine hyperactivity since withdrawal from chronic opiate administration might be expected to have hyperactivity of TIDA neurones in the median eminence and this effect would overwhelm the effect of PRF upon stimulation of PRL secretion (Eidelberg,1976, Lal,1975, Gold et al.,1979). Additionlly, the morphine withdrawal may also effect increment of noradrenergic activity in the hypothalamus which may subjected to show withdrawal symptoms and rebound phenonema (Gold Redmond and Kleber,1978).

Cortisol Levels

High individual fluctuations of serum cortisol levels were evidenced during pre-treatment cycle. Such variations were not mainly due to stressful effect of handling during blood withdrawal because that stressful stimuli were minimized by allowing monkeys to get accustom to such condition for at least 3 months prior to actual study performed. However, these fluctuations were not different in statistics during various phases of the menstrual cycle. Overall values during pre-treatment were slighly lower than perviously report in captive cynomolgus monkeys by Rachpiboon(1984) whom directly taking blood samples after allowing animals to sedate with ketamine, but the value may increase to as high as 2000-2700 ng/ml in temporarily trapped adult free-ranging males of the same species (Settheetham and Varavudhi,1989).

Influences of morphine on serum cortisol levels were dose dependent. Significant decline of cortisol levels were more pronounced among high dose of morphine treated groups (1.6 and 3.2 mg/kg/day). The serum levels of cortisol fluctuated and gradually decreased throughout the treatment period. It is noteworthy to point out that the fluctuation of serum cortisol level in one monkey (no.92) whom tolerated to maintain regularity of the menstrual cycle was not as great as other monkeys whom unable to exhibit regularity of the cycle. Moreover, she was the only monkey in 1.6 mg/kg/day morphine treated group whom failed to exhibit spontaneous galactorrhea, although the serum PRL levels were alwals increasing The effects of chronic administration of opiates on hypothalamic-pituitary-adrenal axis

in animal models were very rare and the period of treatment are not comparable with the present study. However, these data are in agreement with Hellman et al.,(1975) and Ho et al.(1977) who found sinificant decrement of ACTH and cortisol levels in chronic heroin addicted patients.

It has been demonstrated in rats that acute and chronic administration of morphine attenuated the stress-induced norepinephrine and dopamine concentration in hypothalamus (Calderini et al.,1978, Tanaka et al.,1983 and Suemaru et al., 1985), presumably by acting on specific receptor of CRF neuron (Morley,1981, Buckingham,1982) and/or directly suppress on pituitary ACTH cells in response to CRF (Del Pozo et al.,1980, Lamberts et al.,1983). However, morphine may directly decrease sensitivity of adrenocortical cells response to ACTH (Lymangrover et al.,1981, Beyer et al.,1986).

Withdrawal of morphine showed gradual readjustment of serum cortisol levels in 0.8-3.2 mg/kg/day treated groups. This was in sharp contrast with the effect on PRL secretion and metabolic turnover. Resumption of normal reproductive cycle with normal serum levels of E_2 and P also took place within 61 days after withdrawal but the cortisol levels hardly readjusted to normal pre-treatment range within 90 days of post-treatment. However, sexual behaviours during the first few cycles after morphine withdrawal remained low similar to the situation found during treatment period. On the other hand, monkeys previously treated with low and moderate doses of morphine (up to 0.4 mg/kg /day) exhibited high reproductive potential. They were capable of exhibitng successful mating with normal pregnancy within the first and second post-traetment cycles in most cases.