CHAPTER II

LITERATURE REVIEW

Morphine is the oldest analgesic agent known to man and its name derives from the Greek, Morpheus, God of Dreams. It may be given by oral route, but since the effect is variable (Iwamoto and Klaassen, 1977; Greene and Huge, 1982), then, it normally given by subcutaneous or intramuscular routes. The intravenous injection can also be employed (Sawe et al., 1981). Its pain modulation is likely to occur at supraspinal and spinal cord sites (Stoelting, 1980). In the treatment of acute pain the official dose is 8-20 mg, but larger doses up to 32 mg may sometimes be required. The analgesic effect reachs its peak about 20 minutes after intravenous injection and about 1.5 hours after intramuscular or subcutaneous injection (Wood-Smith and Stewart, 1964). Furthermore, various medullary areas are also affected by morphine. The respiratory center is depressed and becomes less sensitive to the stimulant effect of carbon dioxide (Adriani, 1970). This effect is detectable even after the smallest effective analgesic doses of morphine and after overdosage it is the cause of death.

When morphine is used for prolonged period, some patients require increasing doses to provide the same degree of analgesia, and tolerance is said to have developed (Snyder, 1975). Such tolerance is called acquired tolerance usually takes 2-3 weeks to

develop on moderate therapeutic doses, but will occur more rapidly if dosage is raised (Gilman et al., 1985). A cross-tolerance could occur to drugs of closely related chemical structure, for example, other phenanthrene derivatives (Wood-Smith and Stewart, 1964). Physical dependence also develops readily and is revealed by the onset of withdrawal symptoms, either on drug withdrawal or by administration of narcotic antagonist. Withdrawal symptoms usually commence about 10-12 hours after the last dose with yawning, sweating and running of the eyes and nose. Then follows a restless irregular sleep for 18-24 hours. The previous signs and symptoms then return accompanied by mydriasis (pupils dilation), gooseflesh (a condition in which the skin is raised up in small points) and cramps (severe pain from the sudden tightening of a muscle which make movement difficult) and later by insomnia (habitual inability to sleep), nausea, vomitting and diarrhea. Signs and symptoms reach their peak in 72 hours and they decline over the next 7-10 days. Considerable loss of weight is found during the 48 hours withdrawal period (Light, 1930). During the withdrawal period tolerance to morphine is rapidly lost, and the syndrome may be terminated at any time by a suitable dose of morphine or related drugs. The longer the period of abstinence the smaller the dose that will be reqiured (Wood-Smith and Stewart, 1964). After successful withdrawal the addict is taking food normally, has regained his weight and presents the picture of well being. If he would now revert to the drug, he should never start with more than 8 or 16 mg of heroin or morphine, larger amounts than this causing toxic symptoms and even death(Light, 1930). There are two types of model which explain opiate addiction. The first model involved a change at the level of the opiate

receptor or closely allied structures, whereas the latter model, one presumes that morphine suppresses some neuronal system in the brain. To compensate, a completely separate system increases its activity to counteract the suppressive action of morphine where upon the organism is tolerant. When morphine is withdrawn, the overactive second system produces withdrawal systems. During addiction, the hypersensitivity to antagonists would be coupled with subsensitivity to agonist (Snyder, 1975).

Traditional opiate antagonists differ chemically from agonists only in the substitution of an N-allyl, N-cyclopropyl or related group for the N-methyl of agonists (Figure 1) (Snyder, Pert and Pasternak, 1974; Snyder, 1975). The antagonists are much more potent than the opiate agonists themselves and small doses of antagonists can reverse the effects of 10 to 100 times greater doses of opiates (Snyder,Pert and Pasternak, 1974).

The Biological Disposition of Morphine

The biological disposition of morphine is composed of the absorption, distribution, biological transformation and elimination processes (Bunet and Sheiner, 1985). In speaking of morphine recovered from biological media, the terms frequently encountered are "free morphine", "bound, combined or conjugated morphine" and "total morphine". Free morphine is generally assumed to be the unchanged parent compound. The terms bound, combined and conjugated have been used interchangeably to indicate the morphine release after acid hydrolysis, and the quantity of bound morphine is estimated by taking

the different between the total and free morphine found in a given sample (Way and Adler, 1961). Approximately one-third of the unchanged morphine found in human (Olsen, 1974) and rhesus monkey plasma (Rane et al., 1984) is bound to albumin with a substantially lesser amount bound to globulin fractions. Protein-bound morphine is pharmacologically inert, and it relates to the fact that only free morphine (i.e., not bound to proteins) is capable of penetrating biological membranes to reach its sites of action, metabolism and excretion. But the protein-bound morphine complex can dissociate rapidly to release free morphine under certain condition (Greene and Hug, 1982).

The rate of increase in plasma levels of morphine as a function of time following oral ingestion or following subcutaneous or intramuscular injection is therefore usually used to estimate its absorption (Greene and Hug, 1982). Morphine is rapidly and completely absorbed after intramuscular as well as subcutaneous injection (Brunk and Delle, 1974). After intramuscular morphine injection in normal man, peak plasma levels are reached within 20 minutes and the absorption half-life averages 7.7 $\pm$ 1.6 minutes (Stanski, Greenblatt and Lowenstein, 1978). The absorption is 90 percent complete within 45 minutes (Wood and Wood, 1982). In monkeys, a level of 6 ug/ml of the free morphine is attained in plasma within 30 minutes after subcutaneous administration of 30 mg/kg and the maximum level of 8 ug/ml occurs after 120 minutes (Mellet and Woods, 1956). Contrary to clinïcal impression, orally administered morphine is rapidly absorbed from the gastrointestinal tract, and a peak plasma level is achieved within 15 minutes (Brunk and Delle,

1974). Peak plasma concentrations of morphine are, however, substantially less following oral administration than they are following subcutaneous, intramuscular, or intravenous injection. This is manifestation of the so-called "first-pass effect" (Iwamoto and Klaassen, 1977). The first-pass effect is due to the fact that morphine is absorbed from the gastrointestinal tract into the portal circulation, a considerable portion of the absorbed morphine is taken up by the liver and metabolized before it reachs the central circulation (Benet and Sheiner, 1985). In rat, 82 percent of morphine absorbed from the gut is metabolized before reaching the peripheral circulation (Iwamoto and Klaassen, 1977). Metabolism of morphine absorbed from the gastrointestinal tract takes place in the intestinal mucosa as well as in the liver (Dahlstrom and Paalzow, 1975). The amount metabolized in each of these two sites has been determined in rats by comparing systemic plasma levels following oral ingestion, following portal intravenous injection, and following systemic intravenous injection of morphine (Iwamoto and Klaassen, 1977; Sawe et al., 1981). Fifty to sixty percent of the first-pass effect is found to be due to metabolism and/or extraction of morphine by intestinal epithelium, 33-50 percent to hepatic metabolism (Iwamoto and Klaassen, 1977).

In pharmacokinetic terms, distribution refers to where a drug goes in the body after it has been absorbed. The rate at which morphine is taken up by an individual tissue or organ depends upon its blood flow, the permeability coefficient of morphine for membrane in that tissue, and the morphine concentration gradient between plasma and the tissue (Greene and Hug, 1982). In general, free

morphine, like most basic amines, rapidly leaves the blood and concentrates in the tissues, particularly parenchymatous tissues (Way and Adler, 1961). Kidney, lung, liver and spleen show a predilection for the drug, with by far the highest concentration being found in the kidney. Certain endocrine glands such as the adrenal, thyroid and pancreas, also appear to concentrate the drug. While the amount of the drug found in the brain is extremely minute (Mellet and Wood, 1956). Extensive cumulation of morphine in tissue does not occur, tissue levels fall to quite low levels within 24 hours after the last administration. Conjugated morphine which has relatively low pharmacological activity is found in highest concentration in organs concerned with its excretion, the kidney and gall-bladder (Way and Adler, 1961).

The metabolism of morphine has been of particular interest because, notwithstanding the fact that the disposition of an appreciable fraction of the administered dose is still unknown. A schematic representation of known and postulated pathways of morphine metabolism *in vivo* is presented in figure 3 (Way and Adler, 1961). It is now known that conjugation with glucuronic acid is a major pathway for the detoxification of morphine in the liver (Wood and Wood, 1982; Bodenham, Quinn and Park, 1989). In man, there are two glucuronides which are eliminated by renal filtration; morphine-3-glucuronide (M3G) is pharmacologically inactive, while morphine-6-glucuronide (M6G) has been shown to be analgesically active when injected intracerebrally and subcutaneously in mice (Bodenham, Quinn and Park, 1989; Sear et al., 1989a; 1989b; Shelly, Quinn and Park, 1989). In the hepatic and systemic veins the peak concentrations of

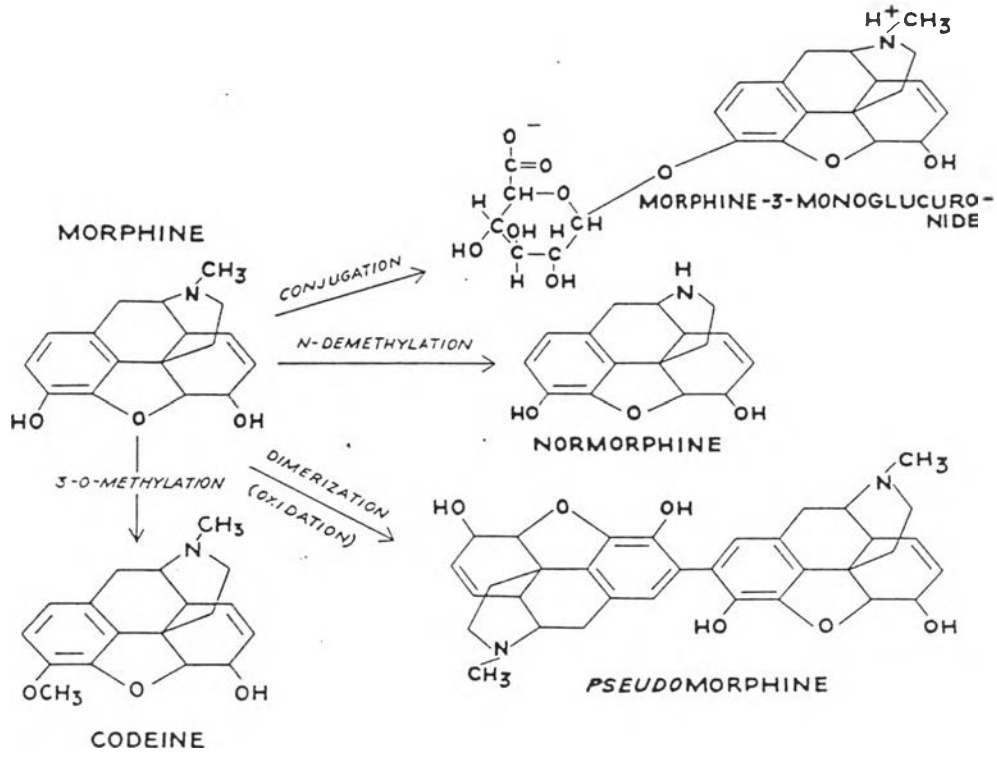

Figure 3 A schematic representation of known and postulated pathway of morphine in vivo (Way and Adler, 1961).

M3G appear later than the peak concentrations of morphine after intravenous administration, and at 120 minutes after dose M3G/ morphine concentration ratio varies between 8 and 11 (Rane et al., 1984). In rhesus monkeys, plasma M6G concentration is very low or unmeasurable after intravenous morphine injection (Rane et al., 1984). It has been reported that cats do not form glucuronides *in vivo* (Robinson and Williams, 1958), presumably because of the lack of glucuronyl transferase, an enzyme for glucuronide formation (Dutton and Grieg, 1957). It appears that the key substance in glucuronide synthesis is uridine diphosphate glucose (UDPG) and that this compound is derived from uridine triphosphate and glucose-1-phosphate (Mills, Lockhead and Smith, 1958). It is quite possible that depletion of carbohydrate stores, which, in turn, limits the amount of UDPG formed, is primarily responsible for the impaired ability to conjugate morphine observed in liver mince obtained from traumatized rats (Goldbaum et al., 1956) or from tolerant rats during withdrawal (Deneau, Woods and Seeves, 1953). In addicts, the amount of morphine destroyed proportional to the amount absorbed (Fry et al., 1929).

Most of a given dose of morphine can be accounted for, largely in the urine and to a lesser but significant extent in the bile and feces. Other fluids, such as saliva, milk, and perspiration, appear to play only a very minor role in the excretion of morphine (Way and Adler, 1961). Ninety percent of the total morphine excretion occurred in the first 24 hours, approximately 75 percent of a dose appears in the urine (Wood and Wood, 1982; Mitchell et al., 1991). Route of administration has no effect on plasma half-life of unchanged morphine, while it alters plasma levels (Brunk and Delle,

1974; Iwamoto and Klaassen, 1977). The elimination half-life ($t_{1/2B}$) is 2.9 hours in human (Stanski, Greenblatt and Lowenstein, 1978), 2.35 hours in rhesus monkey (Rane et al., 1984) and 1.5 hours in adult female cynomolgus monkey (Setheetham, Varavudhi and Yodyingyuad, 1991), respectively. The fraction eliminated bears no relation to height, weight, volume of urine or length of addiction but is directly proportional to the quantity administered (Fry et al., 1929) Mellet and Wood (1956) found that in tolerant rhesus monkeys recieved 15 mg/kg/12 hours morphine showed trends toward a decrease in urinary excretion and increase in fecal excretion.

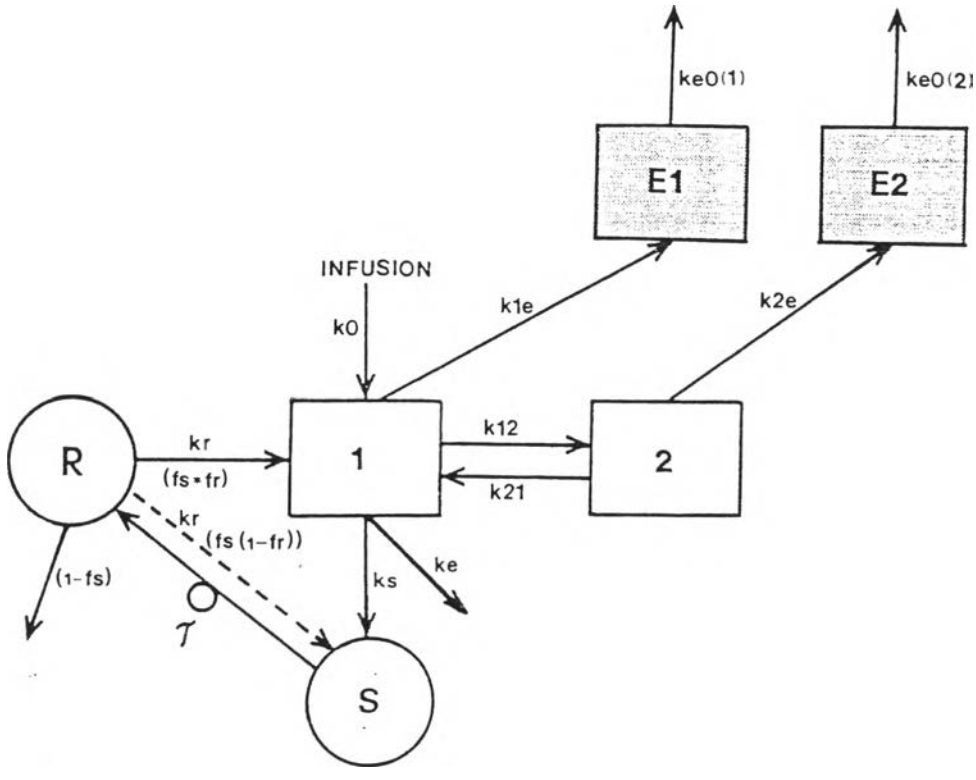

Figure 4 Scheme of the pharmacokinetic-pharmacodynamic model. The pharmacokinetic model is composed of the central(1), pheripheral(2), and storage(S) compartments (gall bladder), the last of which empties completely at time τ into the reabsorption(R) compartment (intestine). A fraction (1-fs) is eliminated in feces, fs is reabsorbed, a fraction fr of this amount is transferred to the central compartment with a rate of kr, and the remaining(1-fr) is transferred directly to the storage compartment. Rate of infusion is k0, intercompartment transfer rates are k12 and k21, rate constant for transfer to the storage compartment is ks, and elimination rate constant is ke. In the pharmacodynamic model, the effect(E1) compartment is driven from the central compartment with the rate k1e and the effect(E2) compartment is driven from the peripheral compartment with the k2e. Exit rates from the effect compartments are denoted keO(1) and keO(2), respectively (adapted from Westerling, Frigren and Hoglund, 1993).

The Effect of Opioid Peptides on Endocrine Functions

The recent discovery of the presence of endogenous opioid peptides in the brain (Stoelting, 1980) and pituitary (Rossier et al., 1980; Wardlaw et al., 1980) has created great interest in their potential physiological functions. Major attention has centered on their antinociceptive (Pert and Yaksh, 1974; Magora et al., 1980; Sangdee, 1982; Pausawasdi, Tontisirin and Bunyaratavej, 1984; Kururattapan and Prakanrattana, 1986; Pramuan and Niruthisard, 1986), behavioral (Cushman, 1972; Jafferys and Funder, 1987) and possible neurotransmitter roles (Frederickson, 1977; Stoelting, 1980). Morover, there is growing in evidence that morphine can also affect release of several pituitary hormones (Morley, 1980; Van Vugt and Meites, 1980). Recent literatures on the effects of morphine and related drugs on endocrine functions are then reviewed here.

Hypothalamic-pituitary-adrenal Axis

It is important to note that opioid systems are not usually tonically active and, hence, opioid antagonists have little or no effect in the state of homeostasis. On the contrary, they are activated by various stressful stimuli, thus allowing them to influence certain effects of such stimuli (Przewlocki, Przeulocka and Lasonl, 1991). It has been reported that B-EP and ACTH were formed from a common precusor proopiomelanocortin(POMC) (Nakanishi et al., 1976; Mains, Eipper and Ling, 1977; Roberts and Herbert, 1977; Mains and Eipper, 1979; Rivier etal., 1982). Furthermore, ACTH and B-EP were released concomitantly after stressful stimuli in rats such as

footshock (Rossier et al., 1977) or leg break (Guillemin et al., 1977). It has been accepted that animals which are submitted to a stressful procedure exhibit antinociceptive response. The antinociception induced by stressful events was linked to the brain endogenous opiates because acute exposure to stress induced changes in level of brain opiate peptides (Akil et al, 1976; Madden et al., 1977) and changes in brain opiate receptor binding characteristics (Chance et al,1979; Christie et al., 1981). Such stress-induced antinociception was found to be fully or partially reverse by naloxone (Chesher and Chan, 1977; Bodnar et al., 1977; Holaday and Belenky, 1980; Grauetal., 1981). The ability of daily administration of naltrexone to reverse the reduction in weight gain induced by the overcrowding of rat was, however, indicative the endogenous opioid peptides may under such chronic stress conditions act, probably indirectly, as suppressors of food intake (Amir, Galina and Amit, 1979). The cross tolerance between morphine and stress-induced antinociception has also been reported (Chesher and Chan, 1977; Spiagia et al., 1978; Drugan et al., 1981; Lewis, Sherman and Liebeskind, 1981; Watkins and Mayer, 1982). It is suggested that B-EP has an unknown peripheral physiological role, perhaps related to changes in adrenocortical function of intermediary metabolism during stress (Trisdikoon, 1983).

Stressful stimuli in adult rats caused the release of immunoreactive CRF (ir-CRF) in several brain areas (Chappell et al., 1986) and increase CRF mRNA and proenkephalin mRNA levels in the paraventricular nucleus (Lightman and Young, 1987), confirming a direct effect of stress on the genomic level of such peptide

containing neurons. CRF is synthesized in paraventricular nucleus of the hypothalamus and is regard as the key peptides involved in the activation of the pituitary-adrenal axis and in the mediation of the endocrine and behavioral responses to stressful and other adaptive stimuli (Sirinathsighji and Heavens, 1989). Its essential role in the pituitary-adrenal response is evident from its potent stimulatory effect on B-EP and ACTH release from the anterior pituitary both *in vivo* (Rivier et al., 1982) and *in vitro* (Buckingham, 1982), and on cortisol release from the adrenal cortex, as well as from its stimulation of sympathoadrenomedullary activity (Antoni, 1986).

Many investigators have been studied the effect of morphine and opioid peptides in human and rat. However, the effect of these substances on the hypothalamic-pituitary-adrenal axis has been conflicted between human and rat. From this different response, the effect of opioid peptides in human will be reviewed separately from the rat.

In general, morphine and opioid peptides administration resulted in adrenocortical stimulation in the rat. In adult rat, morphine and buprenorphine potentiated the elevations in plasma corticosterone caused by injection stress (Bailey and Kitchen, 1987). Since glucocorticoids are established physiological regulators of ACTH secretion. Pretreatment rat with synthetic glucocorticoid dexamethasone before exposured to synthetic CRF totally abolished the increases in plasma ACTH levels and then consequently in corticosterone levels (Rivier et al., 1982). Hence, the diurnal rhythm in response of corticosterone release to opioids was in

relation with the glucocorticoid secretion. The corticosterone release evoked by morphine was demonstrable only in the morning when the basal corticosterone level was significantly lower than in the afternoon (Kiem et al., 1987). Pretreatment of adrenalectomized rats with dexamethasone 2 hours before morphine injection abolished the adrenalectomy-induced sensitization to parenteral morphine (Holaday et al., 1979). The influence of morphine on the functional activity of the hypothalamo-pituitary-adrenocortical system in rats was suggested its effects on the secretion of hypothalamic CRF. Because a single intraperitoneal injection of morphine into the rat caused a rise followed by a fall in hypothalamic CRF content and increase in the concentrations of ACTH in the plasma and adrenohypophysis (Buckingham,1982). In addition, the production of ACTH by pituitary segments *in vitro* was not affected by the addition of morphine to the incubation medium. Whereas, it stimulated the secretion of CRF by isolated hypothalami and was antagonized by naloxone (Buckingham and Hodges, 1979).

From the results in secretion and regulation of ACTH and B-EP are nearly parallel, it may suggest in certain similarities of their functions. Szalay and Stark(1981) considered B-EP activity as an adrenocorticotroph function. They found that B-EP (10^{-4} M) increased corticosterone production of the zona fasciculata and zona glomerulosa cell, but naloxone failed to prevent the stimulatory effect. That means B-EP does not act through the opioid receptors.

In human, Volavka et al.(1979) and Blankstein et al.(1980) reported that naloxone elicited a significant increment in serum

cortisol levels. Moreover, infusion of B-EP significantly suppressed ACTH and cortisol levels in normal human subjects. The decline was in parallel fashion, with both levels falling significantly below control values at dose of 1.0 ug/kg/min B-EP and returning toward control levels 90-120 minutes aftertermination of infusion (Taylor, Dluhy and William, 1983). Taken together, these observations suggest that an inhibitory opioid pathway is involved in the basal regulation of ACTH and that this inhibition is unmarked by naloxone. Since morphine and met-enkephalin (George et al., 1974; del Pozo et al., 1980) did not affect the normal adrenal response to exogenous ACTH and the increase in plasma cortisol after naloxone treatment in men appeared to be preceded by an elevation in plasma ACTH concentration (Volavka et al., 1979). As confirmed the drugs evidently acts at the hypothalamic-pituitary level to inhibit ACTH release. However, a direct effect of morphine on the adrenal gland has not been rule out. The integrated cortisol response to exogenous ACTH was significantly less when the ACTH infusion was preceded by the 30 minutes B-EP infusion than when administered ACTH alone (Beyer et al., 1986). The infusion dose of B-EP raised plasma B-EP levels to over 100,000 pg/ml and it was extremely greater than those normal level (<25 pg/ml). It concluded that very high plasma levels of B-EP may influence the response of cortisol to ACTH through a direct effect on the adrenal cortex. However, even in disease states such as Addison's and Nelson's diseases, such levels of plasma B-EP are not known to be achieved (Beyer et al., 1986).

In heroin-addicted (Ho et al., 1977) and methadone maintenance subjects (Mendelson, Mendelson and Patch, 1975) a

significant depression of ACTH levels were observed whereas no significant reduction of the plasma cortisol levels from the control values. The urinary 17-hydroxycorticosteroids excretion was decreased during addiction to morphine and then followed by tremendous increase in both plasma and urinary 17-hydroxycorticosteroids during withdrawal. These maximal plasma and urinary corticoids did not appear to be related to the duration of addiction but associated with the increase of abstinence point score (Eisenman et al., 1961).

ACTH and cortisol secretion showed a diurnal variation that did not relate to sleep onset and there was a close correlation with B-EP level (Dent et al., 1981). However, the circadian rhythms of ACTH/LPH and cortisol were not due to changes in opiate tone (Grossman et al., 1982). Since inhibition of the endogenous opiate action by naloxone induced elevation of plasma ACTH, LPH and cortisol and the rise above basal levels was consistent, irrespective of whether the infusion was given at 0900, 1800 or 2300 h. Thus, endogenous opiates played a constant tonic inhibition of the pituitary-adrenal axis throughout 24 hours.

Hypothalamic-pituitary-gonadal Axis

According to clinical reports, male heroin addicts experience both diminished sexual drive and impairement of sexual function when actively using narcotics (Cushman, 1972; Cushman, 1973; Martin, 1973). Diminised sexual drive has been characterized as lack of desire for initiation of, and unreceptivity to, sexual interaction. Impaired sexual function has reported as difficulty in

initiating or sustaining penile erection and significant prolongation of ejaculatory time (Mendelson and Mello, 1975). Similarly, in women, the chronic addiction to these drugs is associated with an amenorrhea and other menstrual disorder (Santen et al., 1975). Barraclough and Sawyer(1955) found that morphine administration into the rat on proestrous period, the begining of the critical period for gonadotropin release in cycling rat, completely blocked ovulation. This anovulation was accompanied with the totally deprivation of LH surge and a less extent of FSH surge (Pang, Zimmermann and Sawyer, 1977).

The effects of morphine on LH and FSH release were considerably evaluated at a hypothalamic site. In female, LH responsed to GnRH were not significantly altered by morphine in stalk-section monkeys, eventhough, the administration of morphine resulted in decrease of circulating LH and FSH in ovariectomized monkey (Ferin et al., 1982). These depressant effect of exogenous opiates was competitively reversed by naloxone (Cicero et al., 1976; Cicero, Badger et al., 1977; Gilbeau et al., 1984). Furthermore, naloxone, which itself enhanced GnRH and LH release under most conditions (Quigley and Yen, 1980; Morley, Baranetsky and Wingert, 1980; Blankstein et al., 1981; Van Vugt et al., 1983; Gilbeau et al., 1984), failed to stimulate LH secretion if it was co-administered with a GnRH agonist (Blank and Roberts, 1982) or if the median eminence has been surgically ablated (Panerai et al., 1983); also, GnRH-deficient women failed to respond to naloxone with an increase in LH levels (Blankstein et al., 1981). As in the female subject, there was a lack of direct effect of morphine on testicular androgen

production in hCG pretreated male monkeys (Gilbeau et al., 1984) or in LH-RH pretreated male rats (Cicero, Badger et al., 1977). Additionally, hypothalamic content of LH-RH was increased after acute administration of morphine favoring the hypothesis that release of hypothalamic LH-RH was inhibited (Muraki et al., 1978).

Both acute and chronic administration of morphine significantly depressed serum LH levels approximately 1 hour after subcutaneous injection. This drop of LH levels were 1-2 hours preceded a corresponding drop in testosterone levels (Cicero, Meyer et al., 1976; Cicero,Badger et al., 1977 and Cicero, Bell et al., 1977). Therefore, a reduction in serum LH levels may be a necessary intermediate step in the subsequent reduction in serum testosterone levels. It should be noted also that the peak serum level of morphine occurred 45 minutes after subcutaneous injection in the rat and there was little morphine present in the serum when the reduction of serum LH and testosterone levels were measured (Cicero, Bell et al., 1977). The luteinizing hormone-depleting effect of the narcotics appear to represent a specific action since the (-)isomers of the narcotics were much more effective than (+)isomer (Cicero, Bell et al., 1977). Chronic morphine administration, 75 mg morphine subcutaneous implantation for 3 days, in male rats was markedly reduced the structural and functional integrity of the secondary sex organs (prostates and seminal vesicles) but there was not change in testicular weight (Cicero et al., 1975; Cicero, Meyer et al., 1976). This impairment of the secondary sex organs produced by chronic morphine administration was not due to a direct effect of morphine. Since morphine had no effect on the uptake of subcellular

distribution of testosterone in the secondary sex organs or on the androgen-dependent accumulation of myo-inositol(Cicero et al., 1975). Consequently, it appeared that the testosterone depletion produced by morphine was solely responsible for its adverse effect on the secondary sex organs (Cicero, Bell et al., 1977).

However, it may be unwise at present to rule out a peripheral role of morphine completely. Since 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). Intratesticular and epididymal localization of opioid peptides in long-term hypophysectomized rat (Tsong, Phillips et al., 1982) and presence of a POMC-like mRNA in Leydig cells (Chen et al., 1984) confirmed their endogenous synthesis. The funtional role of opiates in the testis has also been investigated by many investigators. Intratesticular injection of B-EP significantly decreased testosterone response to intraperitoneal LH treatment in the rat and B-EP attenuated LH effects on androstenedione and testosterone production *in vitro*. Thus, testicular B-EP modulated the endocrine function of the testis in adult rats (Chandrashekar and Bartke, 1992). In restrained rat, the plasma testosterone alteration was correlated with a parallel decrease in testosterone and increase of ACTH and B-endorphin-lipotropin in the testicular interstitial fluid (TIF) (Mann and Orr, 1990). Intratesticular administration of naloxone was also caused a significant increase in testosterone levels (Kant and Saxena, 1993) and stimulation of Sertoli cell proliferation and secretion (Gerendai, 1991). This data are in agreement with the previous

report that testicular proopiomelanocortin-derived peptide may modulate testicular steroidogenesis, suggesting that these factors may play an autocrine or paracrine role in mediating the testicular function.

Endogenous opioid peptide may mediate the gonadal steroid negative feed back in the maintenance of GnRH/LH secretion. Many reports showed that naloxone could elevate LH levels only during the late follicular and mid-luteal phase of the menstrual cycle but was not in early follicular phase (Quigley and Yen, 1980; Van Vugt et al., 1983). These evidences show that naloxone-induced increase in serum LH levels is greatest when gonadal steroid levels is high but absent or markedly attenuate in the castrated or low steroid-secretory state. In adult men, the infusions of the pure (nonaromatizable) androgen, 5-alpha dihydrotestosterone which significantly reduces LH pulse frequency and estradiol-17B which significantly suppress LH pulse amplitude are effectively antagonized by opiate receptor antagonist naltrexone. Moreover, the reduction of LH levels by both steroids are also antagonized by naltrexone (Veldhuis et al., 1984). In postmenopausal women the administration of B-EP elicited the prompt release of PRL without concomitant change in LH levels and also naloxone injection had no effect on LH levels (Reid, Guigley and Yen, 1983). This pivotal observation indicates that endogenous opiate systems are functionally coupled to steroid negative feedback control of episodic LH secretion, at least in part, at the level of the hypothalamic pulse generator for GnRH (Adams et al., 1991) and that, therefore, they may be important to the tonic regulation of LH secretion. By

inference, the functional activity of endogenous opioid peptides exert an inhibitory role on GnRH/LH secretion appear to be ovarian steroid dependent (Nakano et al., 1991).

Hypothalamic-pituitary-thyroidal Axis

Similar to cortisol response, morphine effect on hypothalamic-pituitary-thyroidal axis in rodent and higher primates is different one. In the rat, acute and chronic administration of exogenous opiates and endogenous opioid peptides resulted in decrease basal TSH (Muraki et al., 1980; Judd and Hedge, 1982; Mitsuma and Nogimori, 1983a; 1983b; Pechnick, George and Poland, 1985; Elias et al., 1988; del Valle-Soto et al., 1991) and cold-induced TSH release (Muraki et al., 1980; Sharp et al., 1981; Mannisto etal., 1984). The opiate antagonist naloxone blocked the decrease in TSH due to opiate agonist (Muraki et al., 1980; Mitsuma and Nogimori, 1983a; 1983b; Mannisto et al., 1984), but by itself had no effect on basal TSH levels (Judd and Hedge, 1982) and cold-induced TSH secretion (Muraki et al., 1980; Arancibia et al., 1986). The endogenous opioid peptides probably play no role in basal TSH secretion but may be involved in the heat stress-induced fall in TSH since naloxone blocked this decrease in TSH (Sharp et al., 1981; Judd and Hedge, 1982). Morphine decreases plasma TSH levels via a site of action located in the anterior and posterior hypothalamus (Judd and Hedge, 1982; Mannisto et al., 1984; Rauhala, Tuominen and Mannisto,1987). Bilateral electrolytic lesions involving the region of the medial mammillary nuclei of the hypothalamus abolishes the inhibitory effect of morphine on the pituitary-thyroid activity in

the rat (Lomax and George, 1966). The systemic administration of N-methylmorphine chloride, a quarternary analogue of morphine that does not cross the blood-brain barrier, increases the release of GH and PRL and unaffects TSH levels (Pechnick, George and Poland, 1987). This most likely reflects morphine suppression of TRH release from the hypothalamus. Since opiates prevented the cold-stimulated rise in TSH while significantly increasing the TRH content of the medial-basal hypothalamus, probably due to a decrease in TRH release (Sharp et al., 1981; Mitsuma and Nogimori, 1983b). In fact, *in vivo* ir-TRH release from the median eminence rose sharply within 40 minutes in the rat maintained in the cold room (4 ^{o}C) (Arancibia et al., 1986). *In vitro* study showed that specific presynaptic opiate receptors located on TRH nerve endings (Arancibia et al., 1986). The administration of morphine was unable to modulate the TRH-stimulated TSH secretion (Muraki et al., 1980; Sharp et al., 1981). In addition, the *in vitro* hypothalamic TRH release after addition of endorphins was decreased whereas supplementing the incubation medium of anterior pituitary-halves with either endorphins or enkephalins did not modify the medium TSH release. It needs lower dose in B-endorphin than Leu- and Met-enkephalin to inhibit the hypothalamic TRH release. This inhibiting effect was also prevented by naloxone addition (Jordan et al., 1986). This is consistent with the fact that the hypothalamus contains a high level of u-receptors and that enkephalins are less potent than endorphins since their effects are mediated both by mu and kappa receptors (Gilman et al., 1985).

Following to a decrease of TSH secretion, morphine inhibited ^{131}I-labelled thyroid hormone release from the thyroid gland but

pretreatment with morphine did not alter in the increase of thyroid hormone output resulted from TSH administration (George and Lomax, 1965; Lomax, Kokka and George, 1970). As in the domestric fowl, morphine lowered the circulating T_4 concentration while it enhanced plasma T_3 concentration and these effects were completely blocked in the presence of naloxone. Furthermore, naloxone given alone stimulated plasma T_4 concentrations. These results demonstrated differential effects of opiates on T_3 and T_4 concentrations and suggested that opiates tonically inhibited T_4 release and accelerated its rate of peripheral monodeiodination in the fowl (Harvey, Lam and Scanes, 1987).

These results conflict with subsequent data indicating that morphine may have direct effects on the pituitary and thyroid levels. B-endorphin was found to increase TSH secretion from either rat dispersed pituitary cells or pituitary fragments (Judd and Hedge, 1983). But that effect was not prevented by naloxone, suggesting a nonspecific action. In contrast, naloxone was found to increase basal TSH, alpha-subunit and TSH-beta secretion from rat pituitary dispersed cells, however, that effect was not blocked also by human B-endorphin (Cacicedo and Franco, 1985). Freire-Garabal et al.(1992) reported an evidence of opioid binding sites in the rat thyroid gland. A single injection of morphine (1, 5 and 10 mg/kg) in the rat resulted in an increase of serum T_4 and T_3 concentration at 15 and 30 minutes, with a tendency to return to control levels by the 60 minutes. While only 5 and 10 mg/kg morphine suppressed TSH concentration (Tal et al., 1984). In accordance with the previous *in vivo* study, the incubation of thyroid gland with morphine for 2

hours produced a significant increase in T_4 concentration of incubation medium, and resulted in and accumulation of cAMP in the tissue. Naloxone did not change the T_4 release but its incubation with morphine prevented the morphine-induced changes (Tal et al., 1986). These data suggest that morphine may directly exert a short-term stimulatory effect on the thyroid gland with concomitant inhibitory action on the hypothalamo-pituitary TSH system.

With respect to the effect of morphine in the rat, it assumed that the negative feedback effect of thyroid hormone on TRH and TSH secretion may relate to endogenous opioid system (Berglund et al., 1990; Dou and Tang, 1993). Morphine significantly decreased TSH, T_3 and T_4 in control rat but showed no effect on TSH in hypothyroid (Dou and Tang, 1993) and thyroidectomized rats (Berglund et al., 1990). Thyroxin replacement caused a dose-dependent decrease in serum TSH in both morphine and placebo rats; however, TSH was suppressed significantly more in morphine than in placebo rats (Berglund et al., 1990). During the chronic morphine administration (14 days), acute morphine challenges no longer suppressed the TSH secretion, when TSH levels were still low (Rauhala, Mannisto and Tuominen,1988). Thus, it appears that morphine exert its inhibitory effect on TSH secretion by increasing the negative feedback sensitivity to thyroid hormones.

In human, opiates either unaffected on basal TSH levels (Tolis, Hickeyand Guyda, 1975; Reid et al., 1981) or induced an increase in TSH release (Stubbs 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). Pretreatment with naloxone blocked all of these effects (Devilla et al., 1985). However, naloxone alone was unable to affect significantly TSH secretion (Morley, Baranetsky and Wingert, 1980). The increase of TSH by morphine injection was more evident in hypothyroid subject (Devilla et al., 1985), suggesting a dependent upon the basal thyroid levels. Unfortunately, there were no documents about morphine effect on thyroid hormone in human. It has only a report in heroin addicts showing that the plasma thyroxin level is increased (Ho et al., 1977).

The difference of thyroid response between rat and human may link with the circadian hormonal variation and/or neural control mechanism. In human, TSH levels begin to rise in the evenig and peak at 2300 h (Azukisawa et al., 1976) but nocturnal mammals such as the rat shows minimal levels of serum TSH at night (Dou and Tang, 1993).

In the regulation of TSH hormone secretion, the opioid system was evaluated the possible interaction with the other neuron systems (Mitsuma and Nogimori, 1983a; 1983b; Ruzsas and Mess, 1983; Pende et al., 1987). In human morphine, opiate agonist,and clonidine, alpha-adrenergic agonist, increased TSH secretion in greater degree after the administration of the two drugs with respect to the effect of the single drug (Pende et al., 1987). In the rat, the TSH release-inhibiting effect of enkephalinamide was reversed by pretreatment with serotonin synthesis inhibitor, para-chlorophenylalanine(pCPA) or with the central serotonin receptor blocker, metergolin (Ruzsas and Mess, 1983). The inhibitory effect of B-neoendorphin on TSH release was prevented in haloperidol, dopamine antagonist,

pretreatment in the rat (Mitsuma and Nogimori, 1983b). These results furnish evidence in favor inhibitory effect of opiate on the activity of the TRH-TSH-thyroid system by increasing the activity of the central nervous serotonergic and dopaminergic system.

Prolactin and Lactation

Morphine and other endogenous opiates are clearly established to elevate PRL secretion in rat (Spampinato et al., 1979) , non-human primate (Gold, Redmond and Donabedian, 1979; Wehrenberg et al., 1981; Van Vugt, Webb and Reid, 1989) and human (Tolis, Hickey and Guyda, 1975; Stubbs et al., 1978; Reid et al., 1981). The sites of action are notably involved at hypothalamus and pituitary. However, the hypothalamic pathway may be particularly investigated. The infusion of B-EP and Met-ENK into the third ventricle and posterior hypothalamus increased PRL secretion (Rauhala, Tuominen and Mannisto, 1987). Morphine injected to pituitary stalk-sectioned monkey did not alter the PRL concentration whereas in intact monkey showed a pronounced elevation (Wehrenberg et al., 1981). Also, B-EP failed to stimulate PRL in stalk-sectioned monkey recieving estrogen replacement, indicating that estrogen deficiency was not the cause of their failure response but supporting a hypothalamic site of action (Wardlaw et al., 1980). The addition of morphine, Met-enkephalin and endorphins directly into the culture of rat pituitary cell was unable to stimulate PRL secretion (Rivier et al., 1977; Enjalbert et al., 1979a). However, morphine, Met-ENK and B-EP addition into the hemi-pituitaries culture of male rats could block the inhibitory effect of dopamine on PRL secretion and their action

could be antagonized by naloxone. Whereas naloxone itself did not modulate the inhibitory effect of dopamine on PRL secretion directly. (Enjalbert, Ruberg and Arancibia, 1979; Enjalbert et al., 1979). These data confirmed that opiates do not affect the spontaneous release of PRL *in vitro*, but they attenuate the inhibitory effect of dopamine on PRL release. In the rat, systemic administration of N-methylmorphine chloride, a quarternary analogue of morphine that does not cross the blood-brain barrier (BBB), caused an increase in the release of PRL (Penchnick, George and Poland, 1987). Additionally, PRL secretion induced by morphine blocked by both nalmefene-HCL, long-acting analogue of the narcotic antagonist, and its methyliodide analogue which not cross the BBB (Simpkins, Swager and Millard, 1991), suggesting the effect of morphine outside the BBB. However, the basal PRL levels were reduced by only nalmefene-HCL but not by nalmefene methyliodide indicating that basal PRL secretion is influenced by opioid neurons inside the BBB only (Simpkins, Swager and Millard, 1991).

Preceded administration of opiate antagonist naloxone attenuated the PRL response to opiate agonist (Stubbs et al., 1978; Van Vugt, Webb and Reid, 1989). In human subjects, there was no change in serum PRL following naloxone (Morley, Baranetsky and Wingert , 1980; Blankstein et al., 1980). Therefore, opioid pathway may not involve in basal PRL secretion in man during the waking hour. This conflict with the finding in the rat, naloxone given alone produced a small but statistically significant lowering of baseline serum PRL levels, as well as a mild blunting of the stress-induced serum PRL rise. Then the role of endogenous opioid peptides under

physiological condition in the rat may be a minor one (Van Vugt, Bruni and Meites, 1978; Ragavan and Frantz, 1980). In the monkey, however, naloxone produced a significant reduction in basal serum PRL concentrations (Gold, Redmond and Donabedian, 1979; Gosselin et al., 1983; Gilbeau et al., 1985).

There is compelling evidence that PRL secretion from the anterior pituitary is under the tonic inhibition of the dopamine (Diefenbach et al., 1976; Clemens, Sharr and Smalstig, 1980; Ferland et al., 1980; Moore, Demarest and Johnston, 1980). Specific opiate receptors, opioid peptide-containing nerve terminal and cell bodies localize in the hypothalamus. In accordance, the high density of opiate receptors and dopamine containing nerve terminals are in the ventromedial and arcuate nuclei (Kuhar, Pert and Snyder, 1973; Snyder, Pert and Pasternak, 1974; Snyder, 1975). It may suggest that the necessary anatomical connections for an endorphin-dopamine mechanism mediates opiate agonist and antagonist effect on PRL secretion. The ability of morphine and opiate-like substances to enhance the secretion of PRL from the pituitary gland may result, in part, from an inhibition of dopaminergic activity, since either subcutaneous administration of morphine or intraventricular administration of B-EP or (D-Ala^2)-Met-ENKamide, a synthetic enkephalin analogue, led to an 85-95 percent reduction in the concentration of dopamine in the pituitary stalk plasma when compared to vehicle-treated animal (Gudelsky and Porter, 1979). In addition, morphine ions injected iontophoretically in minute quantities into the monkey arcuate nuclei were markedly decrease the concentration of dopamine in hypophyseal portal plasma, thereby

enhancing pituitary gland secretion of PRL (Haskin et al., 1981). This suppression effect of morphine on dopamine release was prevented by pretreatment with naloxone. There was a significant reduction of PRL response in monkeys that received L-dopa, dopamine precursor, 5 minutes before the morphine stimulus (Wehrenberg et al., 1981). Drugs which stimulate dopamine receptors (piribedil, apomorphine or bromocriptine) or increase availability of dopamine (pargyline) inhibit the effect of B-EP on plasma PRL. Whereas, drugs which antagonize dopamine receptors (haloperidol or domperidone) or decrease availability of dopamine (alpha-methyltyrosine) potentiate the effect of B-EP on plasma PRL (Gold, Redmond and Donabedian, 1979; Van Vugt et al., 1979; Van Loon, De Souza and Shin, 1980; Delitala, Grossman and Besser, 1981). Morphine blockes the inhibitory effects of amineptine, a dopamine reuptake inhibitor, on serum PRL release, possibly by decreasing the concentration of dopamine available for reuptake (Van Vugt et al., 1979). Furthermore, B-EP also decreased the rate of dopamine turnover in the median eminence, and increased serum PRL levels approximately 10 folds (Van Vugt, Bruni and Meites, 1978). These neurochemical data support the interpretation that the opiate agonist may interfere with the functional action of dopaminergic system by inhibiting the synthesis, release and turnover of dopamine from the TIDA neurons to increase serum PRL, whereas the opiate antagonist naloxone would augment dopamine activity in the hypothalamus by blocking endorphin-mediated inhibition of dopamine activity (Gold, Redmond and Donabedian, 1979; Gudelsky and Porter, 1979; Van Vugt et al., 1979; Van Loon, De Souza and Shin, 1980; Wehrenberg et al., 1981).

Other hypothalamic neurotransmitters may involved in the mechanisms that permit opiates to increase PRL release, since many agents are present in the hypothalamus that can either inhibit and promote PRL release (Benker et al., 1990). Among the most effective stimulators of PRL release is serotonin (Benker et al., 1990). The rise of PRL levels following morphine is significantly diminished in rat treated with metergoline and cyproheptadine, serotonin receptor blocker, and p-chlorophenylalanine, serotonin synthesis inhibitor (Koenig et al., 1979). 5,6-Dihydroxytryptamine, a neurotoxic drug which destroyed serotonin nerve terminals, almost completely abolished the rise in plasma PRL induced by $EKNH_2$ (an analogue with long lasting analgesic activity of enkephalin) in the rat (Spampinato et al., 1979). However, the serotonergic pathway may not involve in the mechanisms of opiates increase PRL in primates. Since the PRL response in monkey pretreated with methylsergide (a serotonin receptor blocker) 5 minutes prior to morphine stimulation was not different from its control, likewise, the daily administration of p-cholophenylalanine for 6 days failed to alter the PRL response to morphine (Wehrenberg et al., 1981).

B-EP administration induced a prolonged PRL secretion initiated by thyrotropin releasing hormone (TRH) in male rat (Buyden et al., 1987). TRH, a prolactin releasing factor, has a pronounced sex-specific effect on PRL secretion. In male rat has little or no PRL response to TRH (Benker et al., 1990).

The PRL stimulating effect of morphine is mediated by the mu receptors, since a complete suppression of PRL response to morphine

is observed in the rats given intraventricular injection of B-funaltrexamine or intravenous injection of naloxazone, an irreversible, selective and long-acting antagonist of the u_1 receptors, 24 hours beforehand (Spiegel, Kourides and Pasternak, 1982; Koenig et al., 1984). While a preferential delta receptor antagonist compound, ICI 154,129, conspicuously reduced GH secretion and no effect on PRL response to morphine (Koenig et al., 1984). Buprenorphine could interfere with two different, but inter-dependent receptors: at low doses could act at one receptor site (mu receptor), whereas at higher doses could interact with the second lower affinity receptor (kappa receptor) (Amoroso et al., 1988). Therefore, it increased serum PRL levels following the lowest doses and decreased serum PRL levels following the highest doses (Amoroso et al., 1988). On the other hand, the activation of GH probably involves the delta and kappa receptors, since buprenorphine stimulates GH release in a dose-dependent manner and, then, maximum GH release requires higher doses of morphine, mu receptor selective agonist, than those needed for the maximum PRL response (Spiegel, Kourides and Pasternak, 1982).

Prolactin is the most important hormone in the initiation of milk production (Patton and Jensen, 1976). This hormone gradually increases during normal pregnancy and remains high during the establishment of lactation (Austin and Short, 1984). In lactating woman during the first week post partum, the PRL level is elevated to approximately three times higher than normal non-pregnant women, and further episodic increase (up to 100%) are associated with suckling. During the second through sixth weeks post partum, the PRL

level remains approximately twice normal, with a 6 to 20 folds increase with suckling. Twelve weeks after delivery, the serum PRL level is normal, with little or no response to suckling (Tyson et al., 1972). Generally, hyperprolactinemia is associated with hypogonadism and galactorrhea (Koppelman et al., 1984). Galactorrhea, the nonpuerperal secretion of breast milk, may have a myriad of causes and is relatively rare disorder in the male (Volpe' et al., 1972). Galactorrhea is not the inevitable result of hyperprolactinemia, as the establishment of normal lactation it requires the presence of estrogen, progesterone, ACTH, cortisol, insulin and GH as well as PRL (Austin and Short, 1984). Also, as Tyson et al.(1972) reported that a continued elevation of PRL was not necessary for the maintenance of normal lactation. If the other hormonal parameters are not appropiate, a patient with hyperprolactinemia may not have galactorrhea, and a patient with galactorrhea need not have hyperprolactinemia (Quigley and Haney, 1980).

In male patients, hypogonadism has been reported to be often associated with elevated plasma PRL levels. Typically, plasma gonadotropins were often found to be normal, suggesting that PRL might have a direct effect on the gonadal function (Wieland et al., 1967). The action of PRL on testicular steroidogenesis was recently supported by the demonstration of PRL receptors in the interstitial compartment of the testis (Bartke, 1980; Kelly et al., 1989; Klemcke, Amador and Bartke, 1990). The increase PRL levels interfered with the conversion of testosterone into biologically active dihydrotestosterone (DHT) by 5 alpha-reductase, as appeared

from the significant decrease in the ratio of DHT to testosterone (Magrini et al., 1976). DHT has a much higher affinity for the androgen receptor in the target tissues and a much more potent androgen than testosterone (Johnson and Everitt, 1984). Under normal conditions, the major portion of testosterone is converted to DHT, while only a small percentage is aromatized to E_2. When hyperprolactinemia is present with the inhibited conversion of testosterone to DHT, therefore, testosterone is particularly aromatized to E_2 (Prior et al., 1987). Testosterone does not act directly on lactotrophs to increase PRL release. Since there was no increased PRL secretion into the medium when testosterone is added to pituitary cells in culture (West and Dannies, 1980). E_2, on the other hand, can stimulate PRL secretion (Gooren et al., 1988) due to a direct promotion of the PRL secretory mechanism at lactotroph cells (Benker et al., 1990) as well as by an antagonism of the PRL inhibiting effect of DA (Sarkar et al., 1984). Therefore, the treatment of hypogonadism subjects presented with PRL-producing macroadenomas by testosterone could exacerbate the hyperprolactinemia symptom (Prior et al., 1987; Nicoletti et al., 1984).

In puerperal and nonpuerperal women, the initiation of lactation is predicted on a sudden loss of high concentrations of estrogen and progesterone accompanied with an enhancement of PRL level (Tyson et al., 1975; Johnson and Everitt, 1984), owing to the high doses of estrogen and progesterone inhibit the PRL activity at the mammary gland (Turkington and Hill, 1969). In addition, testosterone , a significant sex steroid hormone in normal man,

competitively inhibits normal differentiation of the breast tissue (Archer, 1980). As mentioned previously, suckling is a potent stimulator of transient PRL rise and also causes a rise in B-endorphin-like immunoreactivity (Riskind, Millard and Martin, 1984). Intravenous, bolus administration of B-endorphin produced a PRL response that was similar to the suckling response in terms of latency of onset and duration (Salmanoff and Gregerson, 1986). Salmanoff and Gregerson(1986) reported that the opiate antagonist naloxone suppressed suckling-induced PRL release in a dose-dependent manner and large dose abolished the response. Such finding imply that naloxone blocks a tonic, inhibitory B-endorphinergic input to the tuberoinfundibular dopaminergic neurons occurred during suckling, since a negative correlation between serum PRL and DA in both median eminence and par distalis is always found in the suckling situation (Chiocchio et al., 1979; Selmanoff and Wise, 1981).

Meites, Nicoll and Talwalker(1963) first found that morphine sulfate induced an increase of mammary secretion in estrogen primed rats. Besides in the rat, galactorrhea was also found in female cynomolgus monkeys (Setheetham and Varavudhi, 1993) and male cynomolgus monkeys (Malaivijitnond and Varavudhi, 1993) after a long-term treatment of morphine hydrochloride. However, the report of milk secretion in morphine addicted patients was never seen. Morphine and opioid peptides also inhibit oxytocin secretion and/or milk let-down (Almeida and Pfeiffer, 1991). Oxytocin is the single most important hormone for milk let-down (Greenspan, 1991). It causes a contraction of the myoepithelial cells which surround the alveoli, to induce expulsion of milk into the ducts, with a

consequent build up of intramammary pressure which may cause milk to spurt from the nipple (Johnson and Everitt, 1984).

Opioidergic Activity and the Onset of Puberty

Puberty is a process of complex physiologic and psychologic changes eventual in reproductive maturation. The simply definition of puberty is a period of hormonal secretions, particularly of sex hormones, which lead to increasing bodily (including genital) differentiation of males and females, and which culminates in each individual's ability to reproduce. In males, it means that orgasms are associated with ejaculation of good-quality sperms; in females it means that menstruation (and later ovulation) occurs (Llewellyn-Jones,1987). However, the onset of puberty begins prior to these events; depending on the species, the process may start after only a few days of life or after several years (Almeida and Pfeiffer, 1991). At here, the onset of puberty has been considered as the time when there is an increase in the activity of the GnRH/LH system. This ontogeny of gonadotropin (Gn) secretion during puberty is thought to be initiated by the central nervous system (Chipman, 1980). Terasawa (1992) showed that numerous substances in the brain have been implicated as neurotransmitters and neuromodulator controlling pulsatile LHRH release at puberty. Strong presumptive evidence for these has been drawn from the augmented Gn secretion in both amplitude and frequency that occurs synchronously with sleep in early puberty (Chipman, 1980; Ryan and Foster, 1980). Testosterone levels rise several hours later in boys and suppress Gn secretion, appearently by decresing GnRH pulse frequency. As puberty progress

in both sexes, the peaks of LH and FSH occur more often during waking hour and, finally, in late puberty the peaks occur at all times (Chipman, 1980; Greenspan, 1991). During the peripubertal period of endocrine change prior to secondary sexual development, Gn becomes less sensitive to negative feedback inhibition of sex steroids which underlies increasing FSH and LH output. The latter then drive gonadal steroids production to reach adult levels. This theory of puberty is so-called "Gonadostat" (Johnson and Everitt, 1984). Therfore, before this time a small dose of sex steroids virtually eliminates Gn secretion, while afterward a far larger dose is required to suppress FSH and LH (Greenspan, 1991). Terasawa(1992) found a conflicting result to the gonadostat theory in the female rhesus monkeys that the LHRH neuronal system and pituitary gland were insensitive to the negative feedback effects of estrogen at the prepubertal stage. It first occurred after the onset of puberty in which the developmental changes of input from neuroactive substances (e.g., neuropeptide Y and norepinephrine) increased in pulsatile LHRH release.

A parallel situation on gonadostat theory occurs with opioid control of Gn secretion; during prepuberty, naltrexone, an opiate receptor antagonist, can completely suppress Gn secretion due to its weak opioid effects, while after mid-puberty the anti-opioid effects predominate and Gn secretion increases (Muras et al., 1986). This indicates that Gn secretion is exquisitely sensitive to opiates inhibition in prepuberty or early puberty but less sensitive later. Bhanot and Wilkinson(1983), using FK 33-824, an unusally stable sulfoxide-carbinol derivative of Met-enkephalin which produced

prolonged analgesia even when administered orally (Kream and Zukin, 1979), and the opiate antagonist naloxone in the acutely gonadectomized rat (48 hours), demonstrated a maturation-related reduction in opiate inhibition of LH. Accordingly, physiological pubertal progession may be accomplished by decrease sensitivity of the hypothalamic gonadostat to the inhibitory effects of opioid peptides. Almeida et al.(1987) also reported a transient fall in the inhibitory opioidergic tone upon LH secretion as the normal age of pubertal rats approaches. They found that the inhibitory effects of morphine upon LH secretion in male rats castrated before puberty (26 days old) was progressively decreased for up to 28 days after gonadectomy (age 54 days), but thereafter morphine again caused increasing susceptibility to reduce serum LH values. The minimum inhibition found at 28 days after castration or age 54 days occurred at the time at which male rats normally reach puberty. Cicero et al. (1985) found age- and sex-related differences in response to the opiate agonists and antagonists in the rats. Morphine was no effect on serum LH levels during the first 15 days of ages in male and occurred much latter (30-35 days old) in female, however, naloxone failed to increase serum LH levels during 10-30 days after birth in male and occurred much earlier (10 and 25 days old) in female. The reason for the preceded response to naloxone in female rat may be possibly resulted from the earlier approaching to pubertal stage in this sex (Cicero et al., 1985). Immature prepubertal male rats are markedly sensitive to the effects of morphine on reproductive endocrine parameters than are adult animals since morphine immediately depresses serum LH, testosterone and LHRH levels and the wet weight of the seminal vesicles and testes and prolongs for up to

4 weeks, whereas only transient effect (< 1 week) on some reproductive endocrine parameters (e.g., serum LH and testosterone levels and the weights of seminal vesicles) in adult rats (Cicero et al., 1989).

In human, endogenous opiate antagonist naloxone failed to elevate LH levels during early puberty, suggesting endogenous opiates do not appear to exert a tonic inhibitory influence on LH secretion during this critical time (Fraioli et al., 1984). Whereas naloxone elicited an effective stimulation in LH secretion at adult age (Morley, Baranetsky and Wingert, 1980; Gilbeau et al., 1984). The evidence is in agreement with the progressive increment of plasma B-EP and B-LPH levels during prepubertal stages until pubertal 1 stage of sexual maturation at which time these levels are sustained to adult levels (Genazzani et al., 1983).

Nowaday, the incidence of drug abuse in adolescents has become a matter of increasing concern. Although recent survey suggest that substance abuse, during the past 10 years, has shown a shift toward younger children (Cicero et al., 1989 following Johnson et al., 1987) and, more importantly, substance abuse in this age group remains at extremely high levels. It has been shown that opiates retard sexual maturation (MacDonald and Wilkinson, 1991; Reis and Reis, 1992) and produce physiological and endocrine disturbance (Cicero et al., 1989). Many investigators using non-human primate as an animal model to study the opiates effects, did not consider about the sex and age difference (Gold, Redmond and Donabedian, 1979; Gilbeau et al., 1984; Gilbeau et al., 1985). In

contrast to many studies in women or female animals, only a few studies in males have been done, and most of all did in adults (Gosselin et al., 1983; Malaivijitnond, 1990; Setheetham, Varavudhi and Yodyingyuad, 1991; Setheetham, 1992; Setheetham and Varavudhi, 1993). Moreover, the ramifications of substance abuse during the prepubertal and adolescent periods in human and animals have been explored to only a limited extent. Therefore, the present study of morphine effects in pubertal and adult male cynomolgus monkeys is hopefully to be a paucity of information for futher assessment.

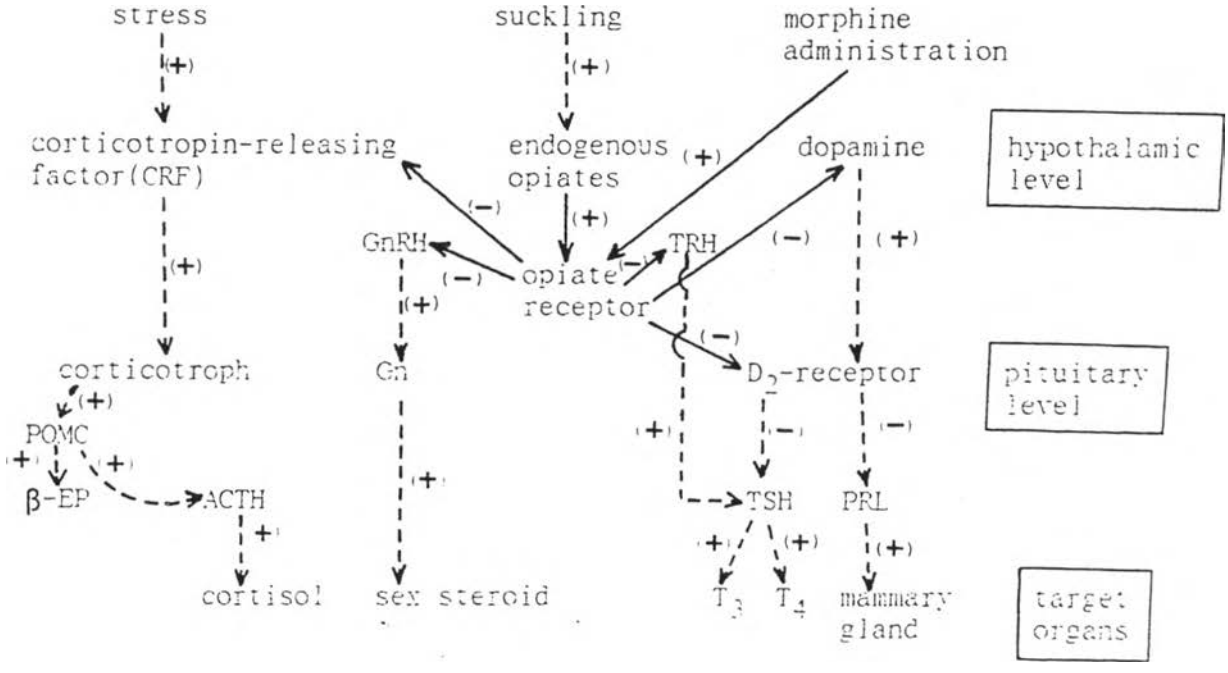

Figure 5 Schematic representation of integral mechanisms of morphine administration, suckling and stress throughout the postulated endogenous opiate pathway on hormonal alteration at target organs. Heavy lines indicated endogenous opiate pathway, dotted lines indicated normal mechnisms. (-) : inhibitory, (+) : stimulatory.