CHAPTER I

INTRODUCTION

In 1806, Serturner reported the isolation of a basic crystalline substance in opium that he named morphine. Morphine is among one of many alkaloids which have a phenanthrene chemical structure. Its constitution in opium is about 3-20 percent by weight (Wood-Smith and Stewart, 1964). Many semisynthetic derivatives could be made by relatively simple modifications of morphine molecule (Way and Adler, 1961; Bavil, 1987). Favorite derivatives for the user are codeine and heroin (Mendelson and Mello, 1975; Findley et al., 1978) (Figure 1). Heroin, or diacetylmorphine, is made from morphine by the acetylation of both the phenolic (C_3) and alcoholic (C_6) -OH groups and it is 4-8 times as potent as morphine (Wayand Adler, 1961; Adriani, 1970).

It is generally believed that any physiological consequences of morphine must first be mediated through the binding of morphine to specific receptors sites (opiate receptors) in the central nervous system (CNS) (Snyder, 1975). Opiate receptor binding appears to be resticted to the nervous tissue (Kuhar, Pert and Snyder, 1973; Pert and Snyder, 1973; Pert, Snowman and Snyder, 1974; Snyder, Pert and Pasternak, 1974; Snyder, 1975). There are dramatic regional variations in opiate receptor binding in the brain of rat, monkey and human, the highest levels are in the anterior amygdala,

periaqueductal grey, thalamus, hypothalamus, caudate head (Kuhar, Pert and Snyder, 1973; Pert, Snowman and Snyder, 1974; Snyder, Pert and Pasternak, 1974; Stoelting, 1980) including pituitary gland (Simantov and Snyder, 1977; Rossier et al., 1980) and pineal gland (Govitrapong et al., 1993). Martin (1967) suggested that complex interactions among morphin-like drugs, antagonists and mix agonist-antagonists could best be explained by postulating the existence of more than one type of receptor for the opioids. Studies the specific binding sites of opioids in brain and other organs have suggested the existence of as many as eight types of opioid receptors. In the CNS, there is reasonably firm evidence for four major categories of receptor, designated mu, kappa, delta, and sigma (Gilman et al., 1985; Kosterlitz, 1991). The mu receptor, which mediates the suppression of the morphine-withdrawal syndrome, supraspinal analgesia, meiosis, euphoria and respiratory depression produced by morphine-like drugs; the kappa receptor, where drugs such as ketocyclazocine (a specific opioid antagonist) produce spinal analgesia but fail to suppress opioid withdrawal; the sigma receptor, is responsible for the pupillary dilatation, respiratory stimulation, dysphoria and symptoms of mania; the delta receptor, bases primarily on its localization in limbic regions of the brain, is thought to be involved in alterations of affective behavior (Wood and Wood, 1982; Gilman et al., 1985). Based on the interaction of each of opioid with any receptors, the opioid could be classified into three groups: morphine-like opioid agonists which are substances acting as agonists primarily at mu and kappa and perhaps delta receptors; opioid antagonists which are substances such as naloxone that are essentially devoid of agonist activity at any receptors; and opioids

with mixed actions that may be category into agonist-antagonists and the partial agonists (Gilman et al., 1985).

Considerable works on opiate action have speculated that opiates may act via altering synaptic transmission in the brain. Pert and his collegues (1974) demonstrated that the specific opiate receptor binding was localized on nerve terminal region of the brain as revealed by its presence primarily in the membrane of synaptosomal fractions of brain hemogenates. For these reasons it is assumed that opiates exert their pharmacologic effects by interacting with selective receptor sites in the neuron. At the cellular levels, opiates have an action that result in increased potassium conductance and/or decreased calcium currents. These implicate in the electrophysiologic effects of the opioids (Gilman et al., 1985). Moreover, morphine has been observed to inhibit prostaglandin E_1 (PGE_1) induced increase in the accumulation of adenosine 3',5'-monophosphate (cyclic AMP or cAMP) in rat brain homogenates (Collier and Roy, 1974) and in culture cell line of neuroblastoma x glioma hybrid (Traber et al., 1975) which coordinated with the membrane potential change (Traber et al., 1974). Minnerman (1977) also reported a potent inhibitory effect of morphine on dopamine stimulated cAMP formation in intact of rat neostriatal slice. The potential mechanism bases on the reduction of adenylate cyclase activity to produce cAMP in the neuron by the specificity of opiod binding interaction. This effect is dependent upon the presence of guanosine-triphosphate (GTP) and is antagonized by naloxone (Traber et al., 1974; Traber et al., 1975). Further evidence that the administration of cAMP antagonizes the analgesic response

of morphine in mice and accelerates the development of torelance to and physical dependence on morphine. Torelance enhancement by cAMP is evaluated by the increased dosage of morphine to produce analgesia and acceleration in dependence by the decrease in dose of naloxone to induce precipitating withdrawal symptom (Ho, Loh and Leong Way, 1973).

When these opiate receptors are discovered, it is wondered why the brain should process receptors that are able to response to exogenous morphine-like substances and it is of interest to speculate that the brain may manufacture its own morphine-like compound. In 1975, Huges and his co-workers isolated two pentapeptides: met-enkephalin (Met-Enk) and leu-enkephalin (Leu-Enk) from pig brain. They exhibit morphine-like actions on the guinea pig ileum and are specifically antagonized by naloxone. From this discovery, it induces researchers to reveal the presence of morphine-like substances in the brain (Bloom et al., 1978) and in the pituitary gland (Rossier et al., 1980). These substances are so-called endogenous opioid peptides. The endogenous opioid peptides are subsequently divided into three distinct families : the enkephalins, the endorphins, and the dynorphins. In endorphins family, generally, pro-opiomelanocortin (POMC) is the important precursor that contained both opioid and non-opioid amino acid sequences (Lundblad and Roberts, 1988; Kosterlitz, 1991). For non-opioid biologically active peptides, it yields ɣ-melanocyte-stimulating hormone (ɣ-MSH), adrenocorticotropin (ACTH), and B-lipotropin (B-LPH) (Lundblad and Roberts, 1988). Within the 91 amino acid sequence of B-LPH is notably found endorphin and B-MSH (Figure 2) (Smith et al., 1983). Indeed, the concomitantly synthesized peptides of ACTH, B-LPH and B-endorphin

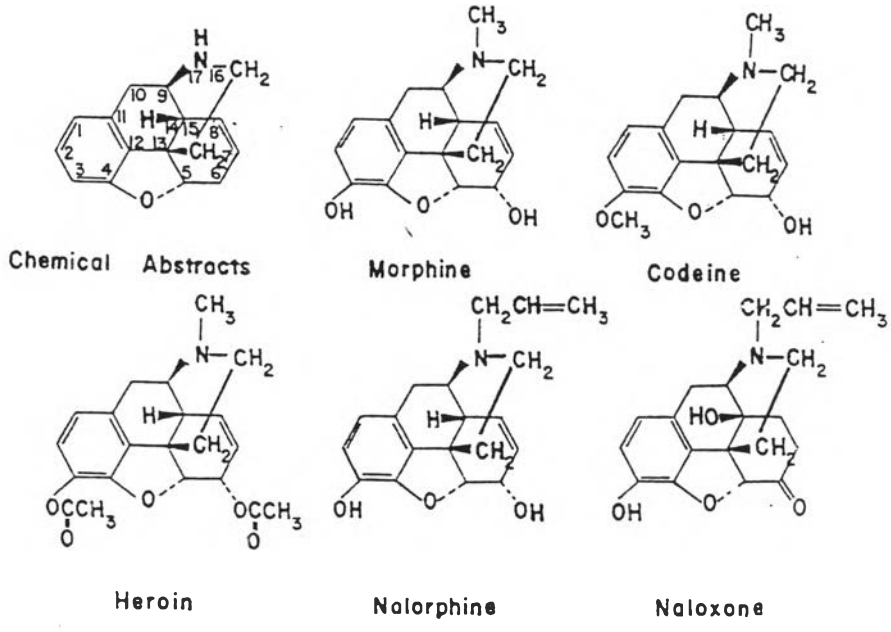

Figure 1 Chemical structure of some of opiate agonists and antagonists(Wood and Wood, 1982).

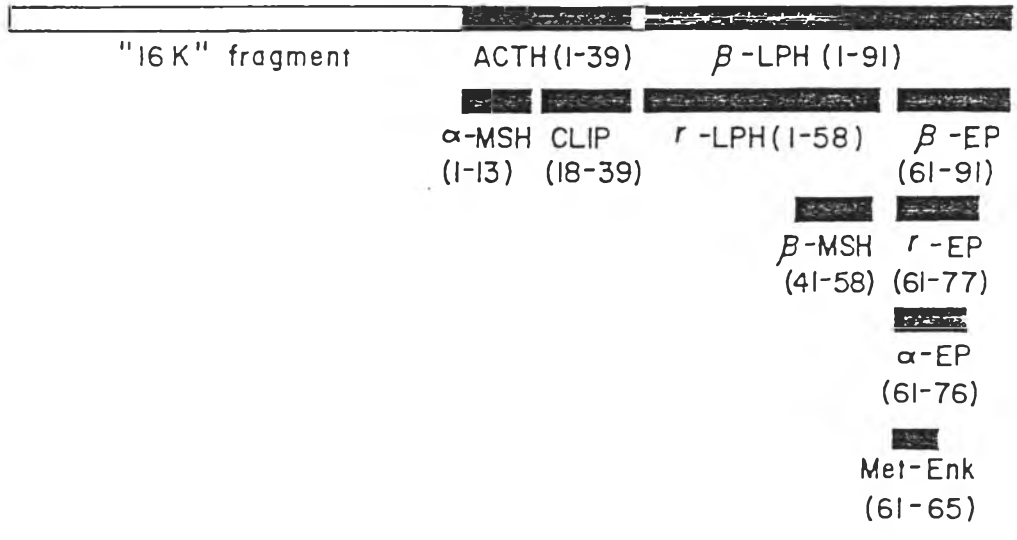

Figure 2 Schematic representation of the structure of the bovine pro-opiomelanocortin precursor and its biologically active peptides(Smith et al., 1983). MSH: melanocyte-stimulating hormone, LPH : lipotropin and CLIP : corticotropin-like intermediate lobe peptide.

(B-EP) *in vitro* are released from the adenohypophysis and increased in amounts in response to acute stress (Millan,1981). By using radioactive labelling of opiate agonist and antagonist such as ^{3}H-dihydromorphine (Kuhar et al., 1973), ^{3}H-morphine, ^{3}H-levophanol (Simantov and Snyder, 1977), and ^{3}H-naloxone (Pert and Snyder, 1973; Snyder, Pert and Pasternak, 1974) show that the distribution of opiate receptors in the nervous tissue have negligible parallel with the opioid peptides activity. The highest concentration of the opiate receptors is in the brain (30 $\pm$ 4 pmol/g of tissue) and the lowest accumulation of opiate receptors is in the adenohypophysis (0.15 $\pm$ 0.1 pmol/g of tissue). Whereas the richest opioid peptide activity can be found in the intermediate pituitary and poorest in the brain (Simantov and Snyder, 1977). The opioid peptide detected in significant amounts in neurohypophysis is enkephalin (Rossier et al., 1980) while in adenohypophysis is B-endorphin (Rossier et al., 1980 and Trisdikoon, 1983). Moreover, high level of B-endorphin is demonstrated in the hypophyseal portal blood (Wardlaw et al., 1980).

These opioid peptides can act directly to the synthesis and release of many neurotransmitters/neuropeptides, for example, somatostatin, dopamine, serotonin, substance P, GABA, and norepinephrine (Reis, Hess and Azmitia, 1970; Lal, 1975; Gudelsky and Porter, 1979; Koenig, 1979; Lee and Wong, 1979; Morley et al., 1980; Morley, 1981; Wehrenberg et al., 1981). Finally, these neuroendocrine substances can affect hypothalamus and/or directly at pituitary gland level (Morley et al., 1981; Wehrenberg et al., 1981). From these results, it may suggest that opiates have a crucial role to modulate the synthesis and release of hypothalamic releasing

hormones and also the pituitary trophic hormones from which the target endocrine glands seem to be the mile stone. It is widely believed that the hormonal effects produced following the administration of morphine or the opioid peptides represent pharmacologic effects whereas hormonal effects observed after the administration of specific opiate antagonists e.g., naloxone, may more closely approximate physiologic effects secondary to alteration in the levels of the endogenous opiates (Van Vugt and Meites, 1980; Morley, 1981).

Opiates increase the hypothalamic content of gonadotropin-releasing hormone (GnRH) favoring the hypothesis that release of hypothalamic GnRH is inhibited (Muraki et al, 1978). Thus the circulating concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) have been reported to be reduced (Cicero, Meyer et al., 1976; Brambilla, Sauhetti and Brunetta, 1977 and Bruni et al., 1977; Ferin et al., 1982). FSH represents a lesser extent than LH to a naloxone reversible supression of opiates (Zimmerman and Pang, 1976). As a result of the decreased concentration of pituitary trophic hormones, the concentration of testosterone and estradiol in plasma decline (Azizi, Vagenakis and Longcope, 1973; Hellman, Fukushima and Roffwans, 1975; Mendelson, Mendelson and Patch, 1975; Mendelson and Mello, 1975; Brambilla, Sauhetti and Brunetta, 1977). Decreased sexual function is frequently encountered in narcotic addicts. Symptoms include loss of libido and impotent, reduction in mean volume of ejaculate and in sperm motility, and regressive changes in Leydig cells in the male (Cushman, 1972; Cicero, Bell and Wiest, 1975; Cicero, Meyer and Wiest, 1975; Crowley

and Simpson, 1978), and anovulation and amennorhea in the female (Gailden et al., 1964; Stoffer, 1968; Wallach, Jerez and Blinick, 1969; Everrett, 1974; Santen et al., 1975). The administration of opiates stimulate prolactin (PRL) and growth hormone (GH) release (Cusan et al., 1977; Kato, Iwasaki and Abe, 1978). Opiates act in the hypothalamus to increase the release of PRL, possibly by reducing the dopaminergic inhibition of pituitary PRL secretion (Tolis, Hickey and Guyda, 1975; Stubbs et al., 1978; Gold et al., 1979; Enjalbert et al., 1979; Melmed, Morley and Kurtzman, 1979; Reid et al., 1981). In rat, the most likely reflected morphine administration is suppression of TRH release and resulted in decreased TSH secretion (Lomax, Kokka and George, 1970; Krulich et al., 1977; 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). Chronic administration of morphine inhibits ^{131}I uptake and ^{131}I-lebelled hormone release by the thyroid gland (George and Lomax, 1965) and decreases the plasma thyroxin levels (Morley, Yamada and Shulkes, 1979). But this effect does not appear to be prominent or consistent in human subjects (Hirooka, Richardson and Prasad 1978; Morley et al., 1980). Opiates inhibit the release of corticotropin-releasing factor (CRF) from hypothalamus (George and Way, 1959) and, therefore, decrease circulating concentration of ACTH in human (Eisenmann, Fraser and Brooks, 1961; Eisenmann et al., 1969; Volavka et al., 1979). But the effect in rat on ACTH and cortisol levels is uncertain (Kokka, Garsia and Elliott, 1973; Ho et al., 1978; Van Vugt and Meites, 1980). With chronic administration, tolerance on hypothalamic releasing factors usually develop. For example, in male patients maintained on

methadone, a potent synthetic analgesic agent, circulating concentrations of cortisol, LH and testosterone are usually maintained within the normal range (Gilman et al., 1985).

Drug abuse is a particular world-wide problem. In Thailand, many worth-while people, especially pubertal men, are the addicted victims (Panupak et al., 1982). Unfortunately, the hormonal determinations have been done in poly-drug abuse patients that could not specify an effect of each drug (Mendelson, Mendelson and Patch, 1975; Cushman, 1972). On the other way, almost all of animal models studied on morphine effects during the past few decades were carried out in rodents which operated with an acute effect on individual hormonal evaluation (Cicero, Meyer et al., 1976; Spampinato et al., 1979; Muraki et al., 1980; Kiem et al., 1987). Only a few recent reports were studied on the chronic effect of morphine in female non-human primates (Settheetham et al., 1991; Varavudhi et al., 1991). Study using male monkeys as an experimental model would fulfill the gap of document between rodent and primates.

In the present study, the alterations of thyroid, cortisol, thyrotropin and major reproductive hormones during pretreatment, treatment and posttreatment periods to morphine hydrochloride together with its pharmacokinetics in male cynomolgus monkeys were investigated. This would have a good opportunity to follow up several hormonal changes during a long-term study in each monkey. Hopefully, these results could provide considerable useful informations of chronic morphine effects upon major endocrine disorders available for future application to the clinical problems.

Aims of the Study

1. To follow up changes in major serum hormones level (PRL, TSH, T_4, testosterone and cortisol) after an acute subcutaneous injection of morphine hydrochloride in pubertal and adult male cynomolgus monkeys.

2. To follow up changes in major serum hormones level in response to chronic morphine treatment and withdrawal in pubertal and adult male cynomolgus monkeys

3. To investigate the pharmacokinetics changes of morphine hydrochloride during the chronic treatment.

4. To investigate some associated changes during treatment period (e.g. B.W., testicular size and galactorrhea)