INTRODUCTION

PAIN

Pain is an extremely complex process that involves the interaction of an array of neurotransmitters and neuromodulators at all levels of the neuraxis (Siddall and Cousins, 1997). Nociception and pain are initiated in the peripheral terminals of primary afferent nociceptors. Nociceptors, including both C- and Aδ- fiber sensory neurons, transduce noxious stimuli of multiple modalities, including mechanical, thermal, and chemical energies, and generate action potentials that are propagated to the CNS (Tanner *et al.*, 1997).

Tissue damage caused by injury, disease, or inflammation releases endogenous chemicals, called algogenic, algesic, or pain-producing substances, in the extracellular fluid that surrounds the nociceptor. These substances include H^+, K^+, serotonin (5-HT), histamine, prostaglandins (PGs), bradykinin, substance P (sP), and many others. They play a causal role in pain associated with inflammation, trauma and a variety of other pathophysiologic conditions. In addition to direct excitatory action on the membrane of nociceptors, these agents may have an indirect excitatory action by altering the local microcirculation. The algesic substances can cause increased capillary permeability and either vasoconstriction or vasodilatation (Raj, 1996).

Location of algesic substances

5-HT, histamine, K^+, H^+, PGs, and other members of the arachidonic acid cascade are located in tissues; kinins are in plasma; and sP is in nerve terminals. Histamine is found in platelets, basophils, and the granules of mast cells; 5-HT is present in mast cells and platelets. Release of these amines may be induced by mechanical injury, noxious heat, radiation and certain byproducts of tissues damage, most notably neutrophil lysosomal materials, thrombin, collagen, and epinephrine. Tissue damage also induces release of lipidic acids of the arachidonic acid cascade; such as the leukotrienes (LTs) and PGs (Raj, 1996).

Bradykinin is a putative inflammatory mediator with a wide spectrum of proinfammatory actions. This peptide activates nociceptive afferent nerves, increases vascular permeability, promotes vasodilation, and induces positive chemotaxis of leukocytes (Hargreaves and Dionne, 1991). Bradykinin is byproduct of the cascade that is triggered by the activation of factor XII of the Hageman clotting system by exposure to negatively charged surfaces such as collagen. This activation results in the conversion of the enzyme prekallikrein to kallikrein, which then acts on the bradykinin precursor kininogen, resulting in the release of bradykinin into the tissues. The action of bradykinin on nociceptors is potentiated by PGs present in the injured tissue compartment (Raj, 1996).

PGs are biosynthesized in the body from certain polyunsaturated essential fatty acids, among which is the abundant arachidonic acid. Arachidonic acid is the precursor of LTs and thromboxanes (TXs), which are qualitatively and quantitatively important prostanoids. Precursor fatty acid normally does not occur free in the cell; it is esterized to phospholipids. Conversion into PGs and release of the related substances thus starts with the liberation of the fatty acid by the action of a phospholipase A, which releases the cell-membrane-derived arachidonic acid. A number of stimuli are known to lead to the activation of phospholipase and thus increased PGs synthesis. These stimuli include norepinephrine and dopamine, which stimulate synthesis of the cellular phospholipids, in part by releasing the noresterified free fatty acid precursors (Raj, 1996).

PGs act via a number of receptors coupled with second messengers but the EP receptor for PGE_2 and the IP receptor for PGI_2 (prostacyclin) are probably the most important for their effects on sensory neurons. PGE_2 stimulated the release of sP from sensory neurons in culture. These depolarizing effects may have due to an increase membrane Na^+ conductance. More usually PGs sensitize sensory neurons, reducing their activation threshold and enhancing their responses to other stimuli (Dray, 1995)

Capsaicin is the major pungent extract from hot peppers of the capsicum family. It has the unique property of selectively activating

polymodal nociceptors following local or systemic administration (Perkins and Dray, 1996). It is a highly potent pain-producing substance, which stimulates nociceptive and temperature-sensitive nerve ending in tissues. It has been shown to cause depolarization of dorsal root ganglion cells associated with C fibers (and probably also Aδ fibers) without affecting other sensory neurons. Capsaicin causes release of substance P (sP) and other peptides from afferent neurons both peripherally and within the spinal cord (Rang and Dale, 1987). Substance P, one of a family of so-called neurokinins, has long been implicated as one of the neurotransmitters involved in nociceptive transmission. Dorsal horn sP has been shown to originate from primary afferent fibers and intrinsic neurons together with a contribution from descending fibers (Dickenson, 1995). It is well established that sP is contained in a large proportion of the C fibers in the dorsal root, and in terminal of these fibers both in the dorsal horn (laminae I and II) and in the periphery. Release of sP from the peripheral nerve terminals of pain afferents probably accounts for the vasodilatation and fluid extravasation that occur in the triple response. Other peptides that occur in different populations of dorsal root ganglionic neurons include vasoactive intestinal peptide (VIP), cholecystokinin, C-terminal octapeptide (CCK8) and somatostatin (SOM). Any or all of these could function as central transmitters, though they are present in fewer cells than sP (Rang and Dale, 1987).

The Pain receptors and their stimulation.

The sensory organs for pain are the naked nerve ending found in almost every tissue of the body. Pain impulses are transmitted to the CNS by two fiber systems. One nociceptor system is made up of small myelinated Aδ fibers, 2-5 μm in diameter, which conduct at rates of 12-30 m/s. The other consists of unmyelinated C fibers, 0.4-1.2 μm in diameter. These latter fibers found in the lateral division of the dorsal roots and are often called dorsal root C fibers. They conduct at the low rate of 0.5-2 m/s. Both fiber groups end in the dorsal horn; Aδ fibers terminate primarily on neurons in laminas I and V, whereas the dorsal root C fibers terminate on neurons in laminas I and II. There is abundant evidence that the synaptic transmitter secreted by primary afferent fibers subserving pain is sP (Ganong, 1997).

Some of the axons of the dorsal horn neurons end in the spinal cord and brainstem. Others enter the anterolateral system, including the lateral spinothalamic tract. A few ascend in the posterolateral portion of the cord. Some of the ascending fibers project to the ventral posterior nuclei, which are the specific sensory relay nuclei of the thalamus, and from there to the cerebral cortex. Many fibers activated by pain end in the reticular system, which projects to the midline and intralaminar nonspecific projection nuclei of the thalamus and from there to many different parts of the cortex. Others project to the hypothalamus, and some end in the periaqueducta gray, an area

known to be concerned with pain (Ganong, 1997).

Although the precise mechanism by which endogenous chemicals participate in peripheral transduction of nociceptive stimuli into nociceptive impulses is not known, these substances may initiate three mechanisms: (1) those that activate nociceptive afferent fibers and produce pain by local application (e.g., bradykinin, acetylcholine, and K^+); (2) those that facilitate the pain evoked by chemicals and physical stimuli by sensitization of nociceptors but are ineffective in evoking pain themselves (e.g., PGs); and (3) those that produce local extravasation (e.g., sP). In addition to these mediators from extraneural sources, sP and other peptides may have a role in influencing the milieu of the peripheral afferent terminals and thus, indirectly, in the transduction of nociceptive information (Raj, 1996).

The chemical that seems to be most painful of all is bradykinin.. Also, the intensity of the pain felt correlates with the local increase in K^+ concentration as well. And it should be remembered, too, that proteolytic enzymes could directly attack the nerve endings and excite pain by making their membranes more permeable to ions (Guyton, 1991).

Drugs used to treat pain

The control of pain is one of the most important uses to which drugs are put. Analgesic drugs fall into five main categories:

1 Opioids (e.g., morphine). Morphine acts on the mu receptor, one of the three opioid receptors mu, delta and kappa. The highest concentrations of opioid receptors in the spinal cord are around the C fiber terminal zones in lamina I and the substantia gelatinosa with lower concentrations found in deeper later. Presynaptic actions of mu and delta agonists, causing a reduced release of the primary afferent transmitters present in C fibers, are produced by the receptors hyperpolarizing the terminals via the opening of potassium channels. It is believed that many of the post-synaptic opioid receptors are located on nociceptive circuitry such as interneurones or on the dendrites of the deep cells penetrating into the C fiber terminal zone. Whatever the case these different mechanism will have overall similar final effects in reducing activity in nociceptive pathways (Dickenson, 1995). Morphine in the brainstem inhibits spinal nociceptive reflexes (Yaksh, 1996).

2. NSAIDs (aspirin and related substances). Prostanoids are synthesized at the site of injury and can act upon the peripheral afferent terminal to facilitate afferent transduction and augment the inflammatory state. To that degree, inhibition of PGs synthesis can diminish that hyperalgesia state and reduce the magnitude of inflammation (Yaksh, 1996). NSAIDs are widely prescribed agents that have been shown to have significant utility in a variety of acute as well as chronic pain states. These agents serve not to alter pain thresholds under normal condition, but to reduce a hyperalgesic component of the

underlying pain state. NSAIDs are structurally diverse but have the common feature in the ability to function as inhibitors of the enzymes cyclooxgenase (COX), the essential enzyme in the synthesis of PGs. Though PGs are normally generated by the constitutive form of cyclooxygenase (COX-I) and serve a number of physiological functions, during inflammation PGs formation is enhanced by the induction of another form of the enzyme i.e. COX-2. NSAIDs owe their analgesic and anti-inflammatory properties to a block of COX enzymes but compounds that select for COX-2 produce analgesia with fewer side effects (Dray, 1995)

3 Local anesthetics (e.g., lidocaine). The systemic delivery of sodium channel blockers has been shown to have analgesic efficacy in a variety of neuropathies, nerve injury pain state and late state cancer. Importantly, these effects occur at plasma concentrations lower than those required to produce a frank nerve block of nerve conduction. The mechanisms of this action are thought to reflect the characteristics of the pain states that are sensitive to intravenous local anesthetics (Yaksh, 1996).

4 NMDA-receptor antagonists (e.g., ketamine). The current thinking on this aspect of drug action is that it reflects upon its action as an antagonist at the glutamate receptor of the *N*-methyl-*D*-aspartate (NMDA) subtype. The NMDA site is thought to be essential in evoking a hyperalgesic state following repetitive small afferent (C fiber) input

(Yaksh, 1996).

5 Alpha$_2$ adrenoceptor agonist (e.g., clonidine). Systemic alpha$_2$ adrenoceptor agonist has been shown to produce a significant sedation and a mild analgesic. Bulbospinal noradrenergic pathways can regulate dorsal horn nociceptive processing by the release of noradrenaline and the subsequent activation of alpha$_2$ adrenergic receptors. This spinal action of alpha$_2$ is mediated by a mechanism similar to that employed by spinal opiates, but the receptor is distinct (Yaksh, 1996).

INFLAMMATION

Inflammation is commonly divided into three phases: acute inflammation, the immune response, and chronic inflammation. Acute inflammation is the initial response to tissues injury; it is mediated by the release of autacoids such as histamine, 5-HT and kinins as well as prostanoids such as PGs and LTs. Those autacoids usually precedes the development of the immune response. The immune response occurs when immunologically competent cells are activated in response to foreign organisms or antigenic substances liberated during the acute or chronic inflammatory response. The outcome of the immune response for the host may be beneficial, as when it causes invading organisms to be phagocytised or neutralized. On the other hand, the outcome may be deleterious if it leads to chronic

inflammation without resolution of the underlying injurious process. Chronic inflammation involves the release of a number of mediators that are not prominent in the acute response such as cytokines from T-cells and complement from antigen-antibody complex (Terr, 1991). One of the most important conditions involving these mediators is rheumatoid arthritis, in which chronic inflammation results in pain and destruction of bone and cartilage that can lead to severe disability and in which systemic changes occur that can result in shortening of life (Katzung and Furst, 1998).

FEVER

A fever is an abnormally high body temperature. The most frequent cause of fever is a viral or bacterial infection (or bacterial toxins). Fever may cause by abnormalities in the brain itself or by toxic substances that affect the temperature-regulating centers. These include bacterial diseases, brain tumors, and environmental conditions that may terminate in heat stroke (Guyton, 1991; Tortora and Grabowski, 1996).

Many proteins, breakdown products of proteins, and certain other substances, especially lipopolysaccharide toxins secreted by bacteria, can cause the set point of the hypothalamic thermostat to rise. Substances that cause this effect are called pyrogens. It is pyrogens secreted by toxic bacteria or pyrogens released from degenerating

tissues of the body that cause fever during disease conditions. When the set point of the hypothalamic temperature regulating center becomes increased to a higher level than normal, all the mechanisms for raising the body temperature are brought into play, including heat conservation and increased heat production. Within a few hours after the set-point has been increased to a higher level, the body temperature also approaches this level (Guyton, 1991).

Experiments in animals have shown that some pyrogens, when injected into the hypothalamus, can act directly on the hypothalamic temperature regulating center to increase its set-point, though still other pyrogens function indirectly and also may require several hours of latency before causing their effect. This is true of many of the bacterial pyrogens, especially the endotoxins from bacteria, as follows:

When bacteria or breakdown product of bacteria are present in the tissues or in the blood, these are phagocytized by the blood leukocytes, the tissue macrophages, and the large granular killer lymphocytes. All of these cells in turn digest the bacterial products and then release into the body fluids, the substance interleukin-1 (IL-1), which is also called leukocyte pyrogen or endogenous pyrogen. The IL-1, on reaching the hypothalamus, immediately produces fever, increasing the body temperature in as little as 8 to 10 min. As little as one ten millionth of a gram of endotoxin lipopolysaccharide acting in this manner in concert with the blood leukocytes, tissue macrophages

and killer lymphocytes can cause fever. The amount of IL-1 causes fever by first inducing the formation of one of the PGs or a similar substance and this in turn acting in the hypothalamus to elicit the fever reaction. When PGs formation is blocked by drugs the fever is either completely abrogated or at least reduced. In fact, this may be the explanation for the manner in which aspirin reduces the degree of fever, because aspirin impedes the formation of PGs from arachidonic acid. It also would explain why aspirin does not lower the body temperature in a normal person, because a normal person does not have any IL-1 in hypothalamus. Drugs such as aspirin that reduce the level of fever are called antipyretics (Guyton, 1991).

ANIMAL MODEL USED IN THE PRESENT STUDY

The writhing response induced in rat or mice by intraperitoneal injection of a noxious agent is commonly used as a basis for testing analgesic activity. The response consists of a wave of constriction and elongation passing caudally along the abdominal wall, sometimes accompanied by twisting of the trunk and followed by extension of the hind limbs (collier, *et al.*, 1968). The latency and duration of writhing response depends on the characteristic of the challenge substances. The substance that has a long latency, such as acetic acid or phenylbenzoquinone, may be supposed to act indirectly by liberating an endogenous substance that excites pain nerve endings. On the

other hand the short latency substance such as acetylcholine and bradykinin may directly excite pain nerve ending (Collier, *et al.*, 1968). The present of anti-writhing response of analgesic drugs in this test was found to be well correlating with clinical result in humans (Taber, *et al.*, 1969).

Tail-flick test is a method determining the central acting analgesic activity of substances. The tail-flick is a spinal reflex, which is modulated by supraspinal inhibitory mechanism. The morphine-like analgesics exert an effect both directly on the reflex arc and through the inhibitory supraspinal mechanism (Harris, *et al.*, 1969). The advantage of this model lies in its selectivity, as all the potent narcotic or morphine-like analgesic show inhibitory activity, as all in this model (Howes, *et al.*, 1969).

The formalin test in mice has an advantage over other frequently used tests as it involves a biphasic response with an early and a late phase representing neurogenic and inflammatory pain and agents can be screened for activity in these two models of pain. The first phase response is believed to represent a direct irritant effect of formalin on sensory C fibers whilst the latter phase response is most likely secondary to the development of an inflammatory response and the release of algesic mediators (Hunskarr and Hole, 1987). Acetic acid-induced writhing model is a highly sensitive pain test (Elisabetsky, *et al.*, 1995).

Ear edema induced in rats by arachidonic acid (AA) or ethyl phenylpropiolate (EPP) was suggested to serve as a more useful model for the testing of anti-inflammatory activity (Young, *et al.*, 1984). This experiment seems to be a useful model for the rapid in *vivo* screening of agents since only a small amount of a test substance is needed. By using both edema inducers (AA and EPP), the mechanism involved can be suggested. LTs are involved in the formation of edema when AA is used as inducer. Application of the AA to the ears of mice produces immediate vasodilatation and erythema, which is maximal at 40-60 min. The onset of edema coincides with extravasations of protein and leukocytes. After 1 h, the edema begin to wane rapidly and the inflammatory cells leave the tissue so that by 6 h the ear have return to near normal except for residual erythema (Young, *et al.*, 1984). Kinins, 5-HT and PGs are released in EPP-induced ear edema (Brattsand, *et al.*, 1982; Young, *et al.*, 1984). The assay has been accepted to serve as a preliminary screening test for anti-inflammatory activity because the response is easily measured (Chang, *et al.*, 1986).

The pyrexia induced in rat by brewers yeast, subcutaneously injected, has been used to determine antipyretic activity of many compounds (Teotino, 1963). The pyrexia reaches its peak at 18 h after induction and the assessment is also made at this period. It has been postulated that many chemical neuromediators are involved in hypothalamic regulation of body temperature. PGE_2 is one of the most

potent pyretic agents known and elevated concentration of PGE_2 has been found in cerebrospinal fluids taken from pyretic patients or animals. The drug that inhibits prostaglandin biosynthesis such as aspirin can reduce the pyrexia (Milton, 1982)

LITERATURE REVIEW OF *OCHNA INTEGERRIMA* MERR.

Ochna integerrima Merr. (Figure 1) belongs to the family Ochnaceae, widely grows in the Southeast Asia. It has various names in Thai but the most popular one is Chang-nao or Chang-noom (Smitinan, 1980). It is a small tree, 3 to 8 m high with plenty of leave. The leaf is simple, alternate, oval shape, 4-7 cm wide and 8-20 cm long (Lekhakul, 1993). The flowers are bright yellows, which can be noticed from a far distance. The fruit is round-shaped, 1 cm diameter, green color and turn black after riping (Pongboonroud, 1971).

In Thai folklore medicine, *O. integerrima* is boiled in water and taken as anthelmintic, antiflatulent and analgesic drug as well as for cheering up mental (Pongboonroud, 1971).

No pharmacological study of *O. integerrima* has been reported to date but there are some studies of plant in the same genus i.e. *O. obsusata*. In India parts of stem and bark of *O. obsusata* are used as anti-inflammatory and analgesic agent. The preliminary study using 90% ethanol extract of *O. obsusata* reveals that it did possess analgesic and anti-inflammatory activities in acetic acid-induced

writhing and carragenin-induced pedal edema models, respectively (Sivaprakasam, *et al.*, 1996).

Figure 1. *Ochna integerrima* Merr., Family Ochnaceae

PURPOSE OF THE SUTDY

The purpose of this study is to evaluate the analgesic, antipyretic, and anti-inflammatory properties of the methanol extracts of *O. integerrima* in comparison with reference drugs, and to compare those activities among them, as well as to find out some mechanisms of action.