CHAPTER I

INTRODUCTION

Pain is a multidimensional sensory experience that is intrinsically unpleasant and associated with hurting and soreness. It may vary in intensity (mild, moderate, or severe), quality (sharp, burning, or dull), duration (transient, intermittent, or persistent), and referral (superficial or deep, localized or diffuse). Although it is essentially a sensation, pain has strong cognitive and emotional components; it is linked to, or described in terms of, suffering. It is also associated with avoidance motor reflexes and alterations in autonomic output. All of these traits are inextricably linked in the experience of pain. (Clifford, 2004)

Pain can be essentially divided into 2 broad categories: adaptive and maladaptive. Adaptive pain contributes to survival by protecting the organism from injury or promoting healing when injury has occurred. Maladaptive pain, in contrast, is an expression of the pathologic operation of the nervous system; it is pain as disease.The sensory experience of acute pain caused by a noxious stimulus is mediated by a specialized high-threshold sensory system, the nociceptive system. (Clifford, 2004)

Nociception

The sensory component of pain begins with the transmission of noxious stimuli from the point of tissue injury or disease to the spinal cord, and then to higher central nervous system (CNS) centers where these signals reach consciousness. Pain, by definition, is a conscious event. Noxious stimuli are called *nociceptive impulses* before they reach the brain. Transmission of these impulses from the site of insult to the CNS is termed *nociception*. At all points where nociception occurs, chemical interactions may enhance or inhibit the transmission of nociceptive information. Only when the noxious signal reaches the level of consciousness is it perceived as "pain." This pain then can be modulated by emotions such as anxiety and depression, other sensory input, and the nociception itself; this can be described as the "emotional" component of pain. (Bradford et. al.,2004)

On the other hand, nociception is initiated by stimuli that activate the peripheral terminals of nociceptors, a highly specialized subset of primary sensory neurons that respond only to intense stimuli. Nociceptors have unmyelinated (C-fiber) or thinly myelinated (A-fiber) axons. The receptive properties of these sensory neurons are determined by their expression of transducing ion-channel receptors, which have a high threshold of activation to external stimuli . Many (but not all) of these transducers have been identified, including those that cause the response to noxious heat (42°C) and cold (10°C) and direct chemical irritants, such as capsaicin (Clifford, 2004).

Transmission

The dorsal horns are divided on the basis of histologic characteristics into laminas I-VI, with I being the most superficial and VI the deepest. Lamina II and part of lamina III make up the substantia gelatinosa, a lightly stained area near the top of each dorsal horn. There are three types of primary afferent fibers that mediate cutaneous sensation: (I) large myelinated Aβ fibers that transmit impulses generated by mechanical stimuli; (2) small myelinated Aδ fibers, some of which transmit impulses from cold receptors and nociceptors that mediate fast pain and some of which transmit impulses from mechanoreceptors; and (3) small unmyelinated C fibers that are concerned primarily with pain and temperature. However, there are also a few C fibers that transmit impulses from mechanoreceptors. (Ganong, 2001)

The sense organs for pain are the naked nerve endings 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 are 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 evidence that the synaptic transmitter secreted by primary afferent fibers subserving fast mild pain is glutamate and that the transmitter subserving slow severe pain is substance

P. (Ganong, 2001)

The synaptic junctions between the peripheral nociceptor fibers and the dorsal horn cells in the spinal cord are the sites of considerable plasticity. For this reason, the dorsal horn has been called a gate, where pain impulses can be "gated," ie modified. For example, stimulation of large-diameter afferent fibers from an area from which pain is being initiated reduces the pain. Collateral branches from the touch fibers in the dorsal columns enter the substantia gelatinosa, and it has been postulated that impulses in these collaterals or interneurons on which they end inhibit transmission from the dorsal root pain fibers to the spinothalamic neurons. The mechanism involved may be presynaptic inhibition at the endings of the primary afferents that transmit pain impulses. (Ganong, 2001)

The more intense the peripheral noxious stimulus, the higher the frequency and the longer the duration of the train of action potentials activated in the nociceptors. High-frequency action potentials result in release of neuropeptides, such as substance P; the neuromodulator brainderived neurotrophic factor; and the fast synaptic transmitter glutamate from the nociceptor central terminals in the spinal cord. The neuropeptides act on G-protein–coupled receptors to produce slow, more sustained synaptic currents than does glutamate, and brain-derived neurotrophic factor acts on tyrosine kinase receptor B to modify membrane excitability. Neuropeptide-mediated slow synaptic potentials, together with activation by glutamate of the *N*-methyl-D-aspartic acid (NMDA) receptor, provides an opportunity for activity- or use-dependent plasticity to occur. At resting membrane potentials, the NMDA receptor ion channel is physically blocked by a magnesium ion so that no current flows if glutamate binds to the receptor; activation of the NMDA receptor by glutamate produces excitation only when this block is relieved by depolarization. One form of activity-dependent plasticity is a progressive increase in the output from dorsal horn neurons in response to closely timed repeated input; this phenomenon, known as *wind-up*, represents an acute form of pain amplification during the course of a train of stimuli and is responsible for the increasing pain experienced in response to closely repeated stimulation of the skin by noxious heat (Clifford, 2004).

Modulation

The gate control theory of pain, proposed in 1965 by Melzack and Wall and later updated, has greatly impacted our understanding of pain mechanism and has promoted research into neurological mechanism that can affect the transmission of nociceptive information and perception of pain. This now well-accepted theory posits that at the time of painful injury, activation of non-nociceptive sensations such as heat, cold, vibration. or touch in the same dermatomal distribution as the painful stimulus reduces the sensation of pain. The pain relief that results from application of heat or cold to injury site, or even the simple act of rubbing an injured body part exemplified this phenomenon. The simultaneous activation of non-nociceptive neurons appears to inhibit the transmission of nociceptive information. The gating mechanism in which activation of non-nociceptive neurons closes the gate on the nociceptive information lessens the pain experience (Bradford et. al.,2004).

The initial gate control theory proposed that this was largely a spinal phenomenon. While the spinal cord plays an important part, other levels of the nervous system also appear to be involved. Central control caused by cognitive influences based on beliefs, fears, and prior experiences can profoundly impact the perception of pain (Bradford et. al.,2004).

Mechanism of analgesic intervention

Systemic opioids work at multiple sites within the CNS including those described for the endogenous pain control system. The periaquaductal gray, the medulla, and the spinal cord are also important sites of action for systemic opioids with activation of the descending pain control system a primary effect. Opioids may be administered directly into the epidural or the intrathecal space allowing the drugs to act more directly on opioid receptors in the dorsal horn of the spinal cord. Percutaneous catheters or implanted pumps are used for chronic pain that has been difficult to control with more conservative approaches, such as systemic opioids (Bradford et. al.,2004).

The α_2 sympathetic agonists can have analgesic effects. Clonidine, first used as an antihypertensive agent, activates antinociceptive mechanisms in the spinal cord.

Stimulation of α_2 sympathetic receptors may produce a similar response to activating opioid receptors. Clonidine can be used as an adjunct or alternative to opioids. However, it inconsistently provided pain relief when opioid analgesia is not feasible or effective. Also, high doses of clonidine can produce drowsiness and hypotension that can be problematic (Bradford et. al.,2004).

The NMDA receptor is a very attractive site for ongoing pharmacologic research. This receptor plays an important role in the development and preparation of neuropathic pain and may play a part in other pain states as well. Unfortunately, available drugs that inhibit this receptor have not been clinically useful. The two presently available NMDA antagonists are the injectable anesthetic ketamine and the antitusive agent dextromethorphan for which the metabolite dextrorphan is actually the NMDA receptor antagonist. Both have major limitations. Ketamine can produce analgesic activity in subanesthetic doses, but side effects including drowsiness, dysphoria, and possibly hallucinations, greatly limit the use of this medication. When dextromethorphan doses are increased sufficiently to antagonize the NMDA receptor, the side effects are usually intolerable. Conotokin, a venom isolated from marine snails has potential as an NMDA antagonist with minimal side effects, but clinical studies are needed to confirm this. This agent is administered spinally to gain access to the receptors and, therefore, is less likely to affect body systems where side effects could occur. NMDA antagonists minimally affect acute pain, but if well-tolerated compounds are discovered or developed, they may become important agents for chronic neuropathic pain (Bradford et. al.,2004).

Epibatidine, a cholinergic agonist first isolated from the skin of a frog, is a potent nonopioid analgesic agent, and even more potent synthetic congeners of this compound have been developed. Their effects are blocked by cholinergic blocking drugs, and as yet there is no evidence that they are addictive. Conversely, the analgesic effect of nicotine is reduced in mice lacking the α_4 and β_2 nicotine cholinergic receptor subunits. These observations make it clear that a nicotinic cholinergic mechanism is involved in the regulation of pain, though its exact role remains to be determined (Ganong, 2001).

The cannabinoids anandamide and palmitoylethanolamide (PEA) are produced

endogenously and bind to CB_1 and CB_2 receptors, respectively. Anandamide has now been shown to have definite analgesic effects, and there are anandamide-containing neurons in the periaqueductal gray and other areas concerned with pain. When PEA is administered, it acts peripherally to augment the analgesic effects of anadamide (Ganong, 2001).

In the course of cloning the μ, κ, and δ opiate receptors, an orphan receptor, ORL_1 (for opioid-like receptor 1), that did not bind any of the opioids with high affinity was identified. The natural ligand of this receptor has now been identified. It is a 17-amino-acid polypeptide that resembles dynorphin-17. However, upon intracerebral injection in experimental animals, it causes hyperalgesia rather than analgesia. On that basis, it has been named nociceptin. Nociceptin and its receptor are present in many areas in the CNS, including the hypothalamus, brain stem, and dorsal horn. The nociceptin precursor protein also contains nocistatin, a polypeptide that antagonizes the effects of nociceptin. It is unclear how the polypeptides interact, but it seems likely that they play a role in pain transmission (Ganong, 2001).

BACKGROUND AND RATIONALE

Acute pain, an unpleasant sensory and emotional experience associated with actual or potential tissue damage as defined by International Association for the Study of Pain (IASP), caused by noxious stimuli including chemical, thermal or mechanical. Thus acute nociception generally elicits a protective or defense response to a noxious stimulus. Inflammatory pain is associated with tissue damage and a resultant inflammatory response. It may be regarded as a mechanism to avoid contact with the area to allow healing to occur; thus there may be an advantage to its presence. It is no longer present once the inflammation has subsided.

As a result of adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all the cases. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs (Kumara, 2001). Analgesic drugs are very effective for relieving pain. Since the prices of the available drugs are very expensive and some drugs might have serious side effects associated with their use, investigation of new drugs from natural products may be useful and reduce these problems. Many parts of trees are never been used, such as leaf, bark, or roots. Therefore, if we can use those parts of the trees to produce new drugs, we might be able to have new drugs at a lower cost.

There are many researches reported about investigating new analgesic drugs from natural products, for example, Otuki et al. (2001), presented that ether fraction and triterpene isolated from resin of *Protium kleinii* had an analgesic effect in acetic acid induced writhing and produced significant inhibition of the neurogenic nociception caused by topical injection of capsaicin, but failed to produce analgesic effect in the tail-flick and hot-plate test. In Thailand, there are also several reports about analgesic and antiinflammatory effects of Thai's herbs. For example, in 2002, Janjuree Kaopinpruk

reported that the extract of *Anacadium occidentale* leafs had analgesic activity in mouse hot-plate test, tail-flick test and rat paw-pressure test. In India, Singh et al., (1984) reported that the crude extract from *Cissus quadrangularis* has analgesic effects compared to that of Aspirin in Haffner tail clip and Eddy's hot-plate methods. These studies are therefore designed to examine in other various animal models the analgesic property of the crude extract from *Cissus quadrangularis* dried stems.

PURPOSE OF THE STUDY

The purpose of this study is to investigate the analgesic activity of the crude extract from *Cissus quadrangularis* dried stems compared with reference drugs and to investigate the possible mechanisms involved.

HYPOTHESIS

The crude extract from *Cissus quadrangularis* dried stems has antinociception property when assumed in thermal and mechanical models of nociception in rodents.

EXPECTED BENEFIT AND APPLICATION

Knowledge from the studies of mechanism and analgesic activity of the crude extract from *Cissus quadrangularis* dried stems may lead to the development of a new analgesic drug from natural sources of Thailand.