CHAPTER 2

LITERATURE REVIEW

Kaempferia galanga L. (Zingiberaceae)

The genus *Kaempferia*, a native of India, comprises more than 60 species (Chithra *et al*., 2005) while in Thailand, 15 species are now recognized (Sirirugsa, 1992). *Kaempferia galanga* L., (*K. galanga*). Zingiberaceae family, which Thai local name is " Proh Hom" (Figure 1) has distribution from tropical Africa to India, and throughout Southeast Asia. The center of distribution is in Southeast Asia . The greatest concentration of genera and species is in the Malaysian region (Indonesia, Malaysia, Singapore, Brunei, Philippines and Papua New Guinea). This plant is perennial rhizomatous and small herb with 2-5 centimeters tall. The leaves that mostly 2, flat on ground, 6-14 centimeters long, 5-14 centimeters wide, upper surface glabrous and under surface softy hairy. The flowers are white with a purple spot on the lip. All parts of the plant are aromatic smell. It is the important of natural resourses that provide many useful products for food, species. medicines, dyes and perfume (Sirirugsa, 1997).

K. galanga grows in damp and humid shady places. It is distributed through many parts of Thailand. Other Thai name besides Proh Hom (Central part) are Wan Hom (Northern part) and Proh (Southern part). It has been commonly used as medicinal plant and spices in Thailand (Zaeoung *et al*., 2005) and the aromatic essential oil from the rhizome is valuable to perfumery (Chithra *et al*., 2005)

There have been reported of using *K. galanga* in folk medicine, but not supported by clinical data such as anti-asthmatic, antibacterial, sore throats, fever, swelling (boils), rheumatism, removal of dandruff or scales, anti-inflammation, sore eyes, tonics, toothache, cough and cosmetics and flavoring. The method of preparation as remedy to use in folk medicine included the powdered rhizomes which are applied to the abdomen after childbirth to eliminate excessive air in the body's system. Leaves are rubbed on swollen breasts after childbirth. The rhizome is boiled and drunk as a tonic for health. The underground stem is used as a stimulant

and for treating toothache, chest pains, headaches and constipation. The rhizome has expectorant and carminative properties. The leaves and rhizome are chewed for relieving cough and sore throats. The leaves are also used for making lotions (Ibrahim and Rahman, 1988; Mustafa *et al*., 1995; Othman *et al*., 2006).

Today, many medical plants are believed to be one of important sources of new chemical substances which have potential therapeutic effects. *K. galanga* is a medical plant used extensively for treatment of various disorders including hypertension, rheumatism and asthma (Zakaria and Mustafa, 1994). The rhizomes of *K. galanga* have been used to treat many conditions by the people of various regions where it is found. The most common indications include rheumatism, asthma, headaches, cough, toothaches and use as a poultice for the application on bruises and wound (Perry and Metzger, 1980). According to the traditional medicine, the rhizome of the plant which contains essential oils, have been used in a decoction or powder for indigestion, cold, pectoral and abdominal pains, headache and toothache. The rhizome is also used as stimulant, expectorant, carminative, and diuretics. Rhizomes posseses comphoraceous odour, and decoction of the rhizome is used for dyspepsia, headache, and malaria. The pharmacological activities of *K. galanga* have been reported such as anti-inflammatory activity but no detail of scientifically systematic data were reported (Sadikun, 1987), smooth muscle relaxant (Hidir and Ibrahim, 1991) and vasorelaxant effect (Mustafa *et al*., 1996; Othman *et al*., 2006). Its alcoholic maceration has also been applied as liniment for rheumatism (Keys, 1976; Lieu, 1990; Kanjanapothi *et al*., 2004). The rhizome extract is useful to cure skin diseases, wounds, spleen disorders, cough, and pectoral affilictions, relieve irritation produced by stinging caterpillars. It is also applied externally for abdominal pain in women and used topically for treatment of rheumatism (Hirschhornn, 1983), fungal derived-skin diseases as well as eczema (Tungtrongjit, 1978). In Chinese medicine, *K. galanga* rhizomes has been used as an aromatic stomachic and also as incense (Kanjanapothi *et al*., 2004). In Thailand, the dried rhizome has been used as cardiotonic and CNS stimulant (Mokkhamit *et al*., 1971; Tewtrakul *et al*., 2005).

A

B

C

D

Figure 1. *Kaempferia galanga* L. (A), leaves (B), flower (C), Rhizomes (D)

Chemical constituents

Nakao and Shibu (1924) reported that the rhizome of this plant consists of cineol, borneol, 3-carene, camphene, kaempferol, kaempferide, cinnamaldehyde, *p*-methoxycinnamic acid, ethyl cinnamate and ethyl *p*-methoxycinnamate. The ethyl *p*-methoxycinnamate was reported to inhibit monoamine oxidase (Nero *et al*., 1983).

Puthan and colleages (1926) reported that the oil of *K.galanga* from India possesses ethylcinnamate and ethyl-*p*-methoxycinnamate as the main components.

Anonymous (1959) reported that the rhizome extract contains *n*-pentadecane, ethyl *p*-methoxycinnamate, ethyl cinnamate, Δ^3-carene, camphene, borneol, cineol, *p*-methoxystyrene, kaempferol and kaempferide.

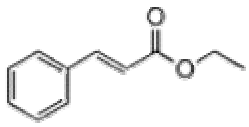
Tanasiriwattana and colleagues (1997) identified the composition of the essential oil of *K. galanga* rhizome by Gas chromatography and Mass spectrometry and found that the rhizome contains (z)-ethylcinnamate (46.60%), 1,8 cineol (17.40%) and -3-carene (11.19%).

Sodibyo (2000) reported that the main components of *K. galanga* oil were *β*-phyllandrene, α-terpineol, ethylcinnamate and dihydro *β*-sesquiphyllandrene.

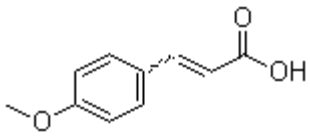
Zaeoung and colleagues (2005) analysed the component of oil of *K. galanga* and revealed the existence of at least 12 compounds (7 of which were identified), in which the main constituent were identified as ethyl cinnamate (61.8%), 3-(4-methoxyphenyl)-2- propenoic acid ethyl ester (18.3%) , *l*-borneol (7.6%), pentadecane (2.6%), terpinene-4-ol (2.0%), *β*-fenchyl alcohol (1.2%) and *p*-cymen-8-ol (1.1%).

Tewtrakul and colleagues (2005) determined the chemical components of volatile oil of dried rhizome using Gas chromatography and Mass spectrometry and found that the major chemical constitutents were identified as ethyl-*p*-methoxycinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%), pentadecane (6.41%), borneol (2.87%), camphene (2.47%), benzene (1.33%), and pinene (1.28%).

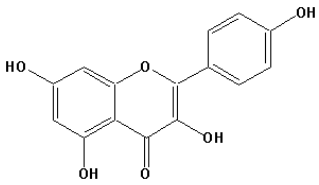
Othman and colleagues (2006) also demonstrated by using of column chromatography (CC) for isolation of bioactive fractions in bio-assay guided study and it was found that the chemical constituents of the active fractions were identified as ethyl cinnamate, undecanone, isopropyl cinnamate, dicyclohexylpropanedinitrile, dipentene dioxide, 9-hydroxy-2-nonanone, (z)-2,7-octadien-1-yl acetate, ethyl cyclohexyl acetate, cis-11-tetradecenyl acetate, 2-heptadecanone, 4-methyl isopulegone, camphidine, ethyl *p*-methoxycinnamate, trans, trans-octa-2,4-dienyl acetate and 10-undecyn-1-ol,3,7-dimethoxycoumarin. The molecular structures of some chemical constituents identified from *K. galanga* are shown in Figure 2.



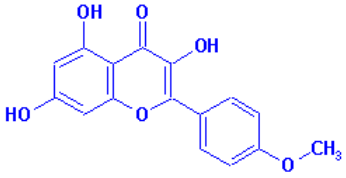
ethyl cinnamate



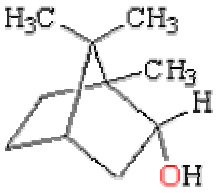
p-methoxycinnamic acid



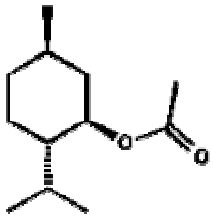
kaempferol



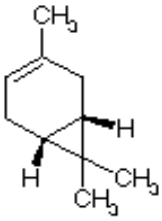
kaempferide



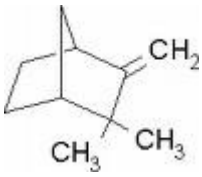
borneol



cineol

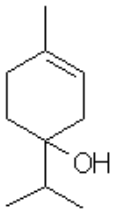


3-carene



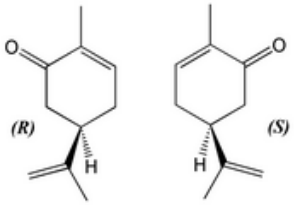
camphene

Figure 2. The molecular structures of various compounds found in *Kaempferia galanga* L.

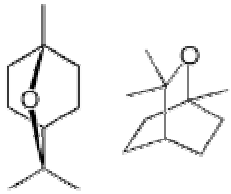


terpinene-4-ol

α-terpineol

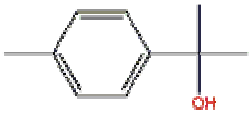


carvone

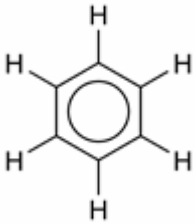


eucalyptol

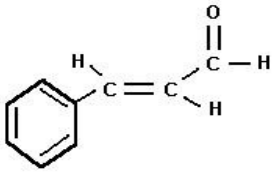
n-pentadecane



p-cymen-8-ol



benzene



cinnamaldehyde

Figure 2. The molecular structures of various compounds found in *Kaempferia galanga* L. (continued)

Pharmacological activities

Sadikun (1987) studies on the effect of water extract of *K. galanga* rhizome on superfused guinea-pig tracheal chain and ileum preparation and mice hind paw edema. The results showed that the extract exhibited anti-inflammatory actions. However, no scientifically systematic data were reported.

Mustafa and colleagues (1996) reported the vasorelaxant effect of chloroform extract of *K. galanga* on the precontracted rat thoracic aorta.

Thomas and colleagues (1996) studies on the amebicidal activity of plants belonging to Zingiberaceae family. The results showed that *K. galanga* was effective against *Streptococcus epidermidis* and *Escherichia agglomerans.*

Achuthan and colleagues (1997) reported the hypolipidemic effect of *Alpinia galanga* (Rasna) and *K. galanga* (Kachoori). The result showed that the oral administration of the extracts (20 mg/day) of both *A. galanga* and *K. galanga* effectively lowered the serum and tissue levels of total cholesterol, triglycerides, phospholipids and significantly increased the serum level of high density lipoproteins (HDL) in high cholesterol fed white Wistar rats over a period of 4 weeks. The results are indicated for various treatment of lipid disorders of these plants especially atherosclerosis.

Tanasiriwattana and colleagues (1997) studies on the chemical composition and antimicrobial activity of essential oil from *K.galanga, K parviflora* and *K. angustifolia.* It has been found that *K. galanga* was able to inhibit the growth of *Staphilococcus aureus, Bacillus sublilis* and *Escherichia coli.*

Chu and colleagues (1998) studies on the amebicidal activity of plant extracts from Southeast Asia on *Acanthamoeba spp* and it was found that the extract exhibited amebicidal activity *in vitro* against three species of *Acanthamoeba* : *A. culbertsoni, A. castellanii,* and *A. polyphaga* that were not lytic for normal macrophage cultures. Similary, the rhizome extract exhibited Epstein-Barr virus inhibitory activity and had no cytotoxicity effect in Raji cells (Vimala *et al*., 1999).

Choochote and colleagues (1999) studies on lavicidal, adulticidal and repellent effects of *K. galanga* and found that the hexane fraction of *K. galanga* exihibited the lavicidal

effect with the LD_{50} of 42.33 ppm. Testing for adulticidal activity, the hexane fraction did not show any promising adulticidal effect. However, it caused a knockdown effect which might be useful as a repellent.

Othman and colleagues (2002) reported that the ethyl cinnamate was isolated from the rhizomes of the *K. galanga*, which showed a vasorelaxant effect by examining on the rat aorta. Ethyl cinnamate inhibited the tonic contractions induced by high K^+ and phenylephrine with respective IC_{50} values of 0.30 ± 0.05 mM and 0.38 ± 0.05 mM. This is possibly due to ethyl cinnamate involed inhibition of Ca^{2+} influx into vascular cells and release of nitric oxide (NO) and prostacyclin from the endothelial cells.

Xue and Chen (2002) studied on the anti-carcinogenic effects of the three compounds in the *K. galanga.* The results showed that both *cis*- and *tran-s* ethyl β-methoxycinnamate has strong anti-carcinogenic potentials.

Ibrahim and colleagues (2003) reported that the essential oil of *K. galanga* showed selective toxicity against *Aspergillrus fumigatus* with a MIC value of 0.63 μg/μL.

Tewtrakul and colleagues (2005) reported that volatile oil of dried rhizome of *K. galanga* exhibited marked antimicrobial activity against Gram-possitive and Gram-negative bacteria and also against a fungus, *Candida albicans* which was stronger than standard antifungal clotrimazole. It is suggested that the essential oil of this plant may be useful for treatment of the diseases caused by these bacteria and fungi such as skin diseases and diarrhea.

Choochote and colleagues (2007) reported that *K. galanga,* exerted repellent potential with median complete protection againt *A. aegypti* times of 0.25 h. These findings exemplified the developing application of repellents of natural origin by reformulation with synergists or additive substances.

Since *K. galanga* has been used as a medicinal plant in Thai folklore medicine, no pharmacological studies *in vivo* have previously been conducted to evaluate the analgesic, antipyretic and anti-inflammatory activities of this plant to comfirm its therapeutic efficacy. In the present study we therefore investigate and report the scientifically systematic data on the antinociceptive, antipyraetic and anti-inflammatory activities and the possible mechanisms of the methanol extract of *K. galanga* in comparison with reference drugs, aspirin and morphine.

Pain

Pain is usually a result of tissue injury in peripheral receptive fields of sensory neurons. Electrical signals generated at these sites are commonly amplified and transmitted further to the higher centers in the central nervous system (CNS) in order to generate a systemic response aimed at self-preservative. Damage to pain-sensing neural elements in sensory pathways (nociceptors) may result in diminished ability to sense pain or in contrast may lead to spontaneous pain and increased pain sensitivity (Coderre *et al*., 1993). A definition of pain provided by International Association for the Study of Pain is " an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in term of such damage" (Papagallo, 2005). Pain is a body defense mechanism and is a warning of a problem, particularly when it is acute (Gould, 2002) and may become chronic where it outlasts any potential for healing and becomes modified centrally (Peck, 2000). This definition takes into account the fact that emotional and evaluative processes come into play and that although tissue damage may not necessarily be taking place, the sensation may feel as such (Campbell, 2004).

Cause of pain

Pain stimuli may occur for many reasons. Pain may be felt because of inflammation, infection, ischemia and tissue necrosis, stretching of tissue, chemicals, or burn. In skeletal muscle, pain may result from ischemia or hemorrhage. Many visceral organs such as the liver, kidney, or brain. In the stomach and intestines, pain may result from inflammation of the mucosa or from distention or muscle spasm. Depending on the cause, pain may sudden and short-term, marked primary by a reflex withdrawal (Gould, 2002).

Pain can be classified in various ways base on duration of pain, type and severity of cause (Kulkantrakorn, 2004). The two types of pain and their qualities has been classified into 2 different major types as fast pain and slow pain

Fast pain is also described by many alternate name such as sharp pain, pricking pain, acute pain, electric pain and others. This type of pain is felt when a needle is stuck into the skin or when the skin is cut with a knife. This pain is also felt when the skin is subjected to

electric shock. Fast, sharp pain is not felt in most of the deeper tissues of the body (Guyton and Hall, 1997). These pain impulses are transmitted over Aδ fibers (Sherwood, 2001).

Slow pain also goes by multiple additional names such as burning pain, aching pain, throbbing pain, nauseous pain and chronic pain. This type of pain is usually associated with tissue destruction. It can lead to prolonged and occur both in the skin and in almost any deep tissue or organ (Guyton and Hall, 1997). Pain impulse originating at nociceptors are transmitted over C-fibers (Sherwood, 2001).

The peripheral pain sensors

The two main types of nociceptor are mechanoreceptors and polymodal nociceptors.

Mechanoreceptors are mainly present in the skin and strong stimulis are pinprick and sudden application of heat (greater than 44 °c). They warn of potential damage and are the afferent part of the withdrawal reflexes. This type of receptor is associated with small myelinated primary afferent neurons designated Aδ (delta). This receptor transmit impulses rapidly. Stimulation of this receptor results in fast pain which occurs almost immediately after injury and is usually sharp, localized and pricking.

Polymodal nociceptors are the nerve endings of unmyelinated primary afferent neurons of the C-type. They are widely distributed throughout most tissues and the classification as polymodal nociceptors indicate that they respond to tissue damage caused by mechanical, thermal or chemical insults. In addition, they respond to chemical mediators formed or released as a result of tissue damage. These impulses are transmitted more slowly than impulses from the mechanoreceptors and travel along unmyelinated C-type nerve fibers. It is these impulses from polymodal nociceptors which are responsible for slow pain that is slower in onset, prolonged, dull, aching, poorly localized and occurs after injury (Park *et al*., 2000) (Table 1). C-fibers comprise around 70% of all nociceptors (Stucky *et al*., 2001).

Table 1. Classification of sensory neurons (Wang, 1995)

Classification		Fiber	Velocity	Propagation
Sensory neuron	Destination	Type	(m/s)	Velocity
Aβ	Laminae II and IV	Myelinated	6-12	35-75
Aδ	Laminae I and V	Myelinated	1-6	5-30
C	Laminae II	Unmyelinated	< 1	0.5-2

Chemical sensitivity of nociceptors

Injury results in the local release of neuron chemicals which mediate or facilitate the inflammatory process. These include bradykinin, prostaglandins, leukotrienes, serotonin, histamine, substance P, thromboxanes, platelet activating factor, protons and free radicals. Some of these chemicals activate nociceptors and therefore are directly involved in producing pain, while others lead to a sensitization of the nociceptor response to natural stimuli and therefore play a role in primary hyperalgesia.

Bradykinin is released upon tissue injury and is present in inflammatory exudates.

Proton is selectively activate nociceptors and produce a sensitization of nociceptors to mechanical stimuli.

Serotonin can also potentiate the pain induced by bradykinin and enhance the response of nociceptors to bradykinin. Mast cells, upon degranulation release platelet activating factor which in turn lead to serotonin release from platelets.

Histamine can lead to a variety of responses including vasodilatation and oedema. Substance P release from nociceptor terminals can cause the release of histamine from mast cells.

Arachidonic acid metabolites (prostaglandins, thromboxanes and leukotrienes) collectively known as eicosanoids. The eicosanoids are generally considered not to activate nociceptors. This sensitizing effect of eicosanoids may play an important role in hyperalgesia associated with inflammation (Wall and Melzack, 1994).

Pain-producing (algesic) substance

Analgesic substances are released by damaged tissues and either directly or indirectly evoke pain. They include substances such as H^+, K^+, acetylcholine, histamine, serotonin (5-HT), bradykinin (Peck, 2000) which can directly stimulate the sensory nerve endings as well as prostaglandins which can potentiate the effect of stimuli but do not directly cause pain themselves. Other, like substance P can increase the permeability of local blood vessel and produce local extravasation.

Sensitization of nerve ending

When an injury is inflicted, pain is first evoked by the stimulation of the nociceptors as well as release of analgesic substances in a localized area. This phenomenon is known as peripheral sensitization. As healing starts, nerve ending of the polymodal C-fibers show increased sensitivity to stimuli. Thus any stimulies in a wider area than the initial site of injury become painful. This process is a result of increases sensitization of nerve endings and is known as central sensitization (Park *et al*., 2000).

Pathophysiology of pain

After experiencing painful stimuli, the chemical messengers released during tissue injury in turn stimulate nociceptors to release neuropeptides such as substance P, neurokinin A, calcium gene-related peptides and nerve growth factor. These neuropeptides, along with catecholamines (e.g., norepinephrine) released from the sympathetic nervous system, lead to peripheral sensitization.

Under the guidance of the sympathetic nervous system after painful injury, the release of norepinephrine stimulates release of excitatory transmitters (e.g. substance P, calcium gene-related peptides and amino acids such as aspatate and glutamate) at the level of the spinal cord. These excitatory transmitters have been implicated in the process of central sensitization and work to prolong the response of dorsal horn neurons, resulting in persistent changes in the excitability of the cell, also referred to " wind-up". Central sensitization refers to sensory changes

in the undamaged tissue surrounding the injury owing to hypersensitive spinal neurons, which produce secondary pain. Tenderness in a zone surrounding the injury is due to central sensitization.

Nociception is conveyed from the periphery to the brain by an adaptable and dynamic pathway. The pathway is transmitted and modulated at three levels: the peripheral nociceptor, the spinal (dorsal horn of the cord), an the supraspinal (brain) (Serpell, 2006)(Figure 3.)

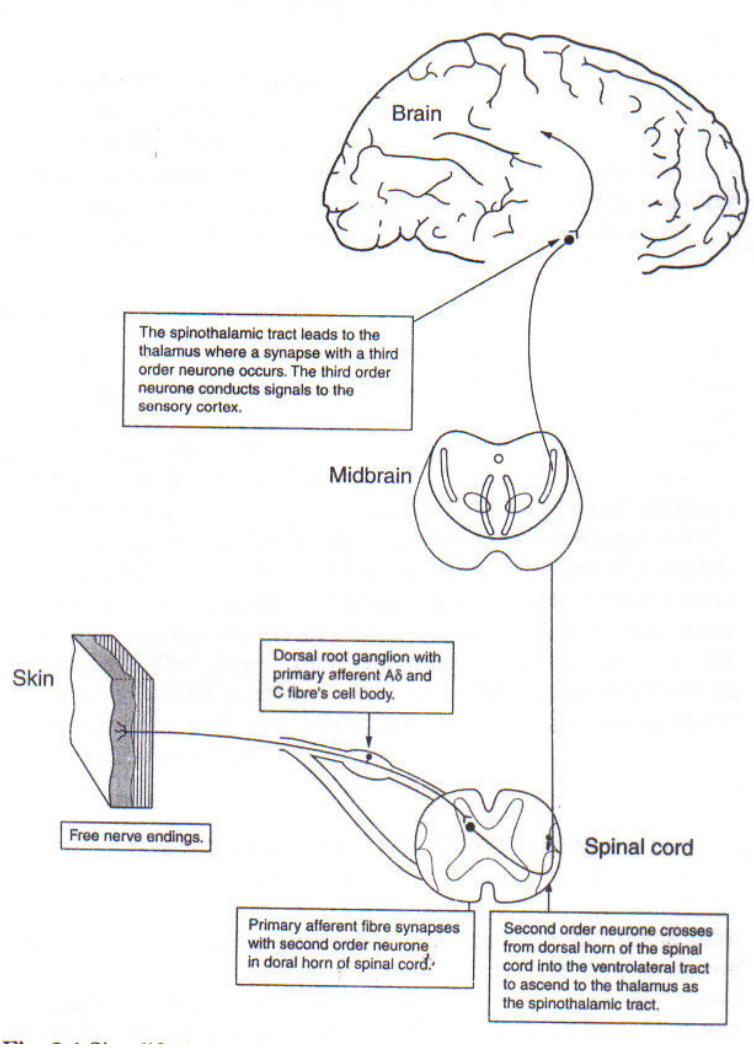


Figure 3. Pain pathway (Park et al., 2000)

Peripheral sensitization

The conduction of nociceptive information from the periphery to the CNS is carried out by neurons that have a bipolar structure, The tip of the peripheral axon lies within the appropriate tissue in which nociceptive information is detected. The cell body lies within the dorsal root ganglion (DRG), with the proximal axon running into the dorsal aspect of the spinal cord. Due to the uneven distribution of ions within and outside the cell membrane, the resting membrane potential of these cells is 50-100 mV, with the positive charge being on the outside of the cell. An adenosine triphosphosphatase (ATPase) pump continuously keeps the concentration of sodium outside the cell at 20 times of that within the cell. Conversely, this pumping system maintains a potassium concentration inside the cell at 35 times of that outside charge becomes either neutral or negative . This result in an action potential, and it can only run in one direction due to the refractory phase that follows membrane depolarization in nociceptors that is from the periphery towards the spinal cord. There are two types of nociceptor : fast conducting Aδ fibers and the more slowly conducting C fibers.

Cutaneous Aδ fibers are myelinated so these fibers are rapid in transfer of information from the periphery to the spinal cord. These fibers are high-threshold receptors which respond to mechanical stimulation such as firm pinch. Some of the fibers also respond to noxious heat (> 45 $^{\circ}$C). Aδ fiber activation results in sharp pain.

The C fibers do not have myelin sheath. They conduct much more slowly than the Aδ fibers. These receptors are sensitive to chemical and thermal stimulation and are frequency refered to as polymodal nociceptors. Overall, the C-fiber population is responsible for 80% of the nociceptive primary afferents and the perception of this information tends to be associated with poorly localized, aching and burning pain.

When the tissue injury occurs, potassium and kinins are released from the damaged cells. These substances stimulate the receptor directly, resulting in the release of neuropeptides such as substance P from the receptor. This in turn causes the degranulation of adjacent mast cells with the production of platelet-activating factor (PAF) which in turn releases serotonin from the platelets. Histamine is also released from the mast cell, starting an

inflammatory reaction within the tissues with vasodilatation, lowered pH and the release of ecosanoids such as leukotrienes and prostaglandins (PGs) (Campbell, 2004) (Figure 4).

Central sensitization

The dorsal horn of the spinal cord is the site where complex interconnection occur between excitatory and inhibitory interneurons and the descending inhibitory tracts from the brain. The second order neuron are of two types: noceptive specific (in laminae II and III), which respond selectively to high threshold nociception or wide dynamic range or convergent neurons (in laminae V and VI), which respond to the range of inputs (Serpell, 2006).

Nociceptive formation within the Aδ and C fibers arrives at the dorsal horn of the spinal cord via the lateral part of the dorsal root. The dorsal root ganglion contains the cell bodies of both types of nociceptor, together with other sensory neurons such as Aα and Aβ fibers. Most C fibers terminate in the superficial dorsal horn at the laminae II (also known as the substantia gelatinosa). Aδ fibers terminate in the laminae I and V. Aδ fibers terminate mainly in laminae I and V with some of their high-threshold fibers ending directly in laminae II. Most cutaneous C fibers terminate in the laminae II. However, visceral C fibers terminate in the laminae I, II, IV , V and X (Campbell, 2004).

Peripheral nerve injury leads to an increase in the general excitability of multireceptive spinal cord neurons (wide-dynamic range neurons with multiple synaptic inputs from the nociceptive as well as the non-nociceptive system). This hyperexcitability in the manifested by increased neuronal activity in response to noxious stimuli, expansion of neuronal receptive fields and spread of a spinal hyperexcitability to other segments (Baron, 2006).

Spontaneous activity of C fibers results in increased dorsal horn excitatory. C fibers release not only glutamate but also substance P, which acts through the neurokinin-1 (NK_1) receptor to increase dorsal horn intracellular calcium and enhance N-methyl-D-aspartate (NMDA) sensivity to glutamate. This up-regulation of glutamate signaling is one aspect of central sensitization (Singleton, 2005). When glutamate release from primary afferent neuron and bind to NMDA, there is an influx of Ca^{2+} into the postsynaptic neuron (Basbaum *et al*., 2005). The resulting influx of Ca^{2+} could activate enzyme such as nitric oxide (NO) synthases or trigger other long lasting cellular changes, so signal transduction coming to sensory projection fields in

the cortex (postcentral gyrus). This part of the cortex, together with the thalamus, is responsible for the conscious perception of pain and particularly localizing and registering the intensity of the pain. The ascending reticular activating system has an influence on evaluation. The limbic system is responsible for emotional reactions triggered by pain while autonomic reactions are controlled by the hypothalamus (Mutscheler and Derendorf, 1995) (Figure 5)**.** This state of hyperexcitability is called central sensitization.

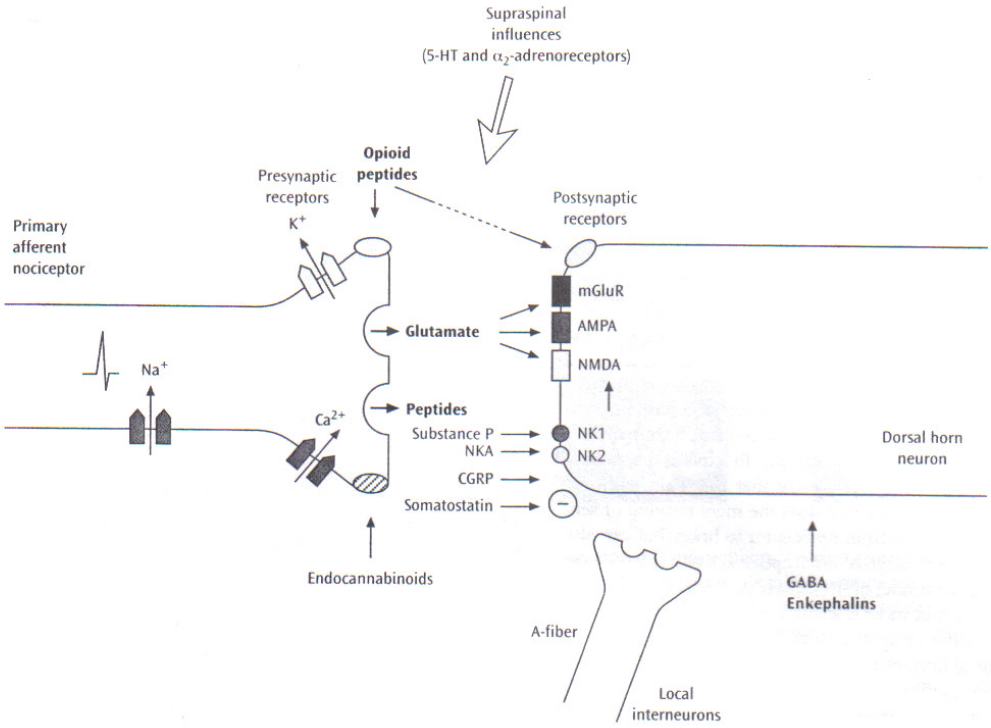


Figure 4. Pain pathway in peripheral sensitization (Rowbotham and Macintyre, 2003)**.**

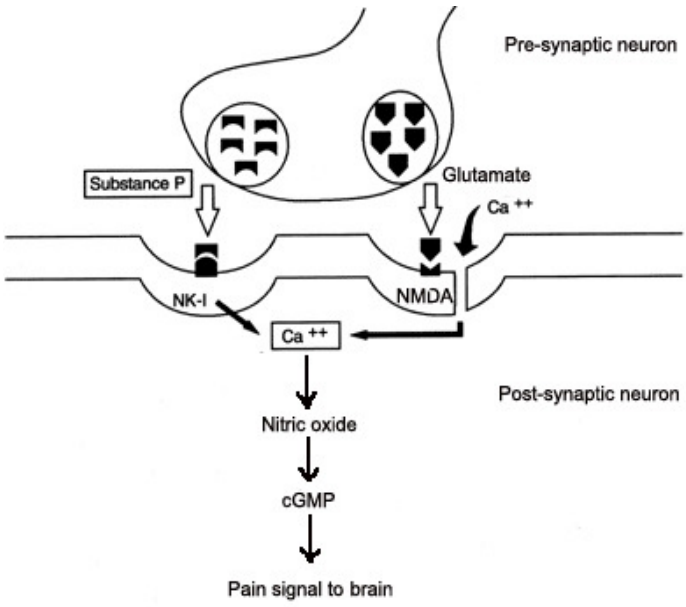


Figure 5. Pain pathway in central sensitization (DeVane, 2001).

Regulation of body temperature

The human body also has a physiological system of thermoregulation. The relatively constant and high body temperature frees biochemical reactions from fluctuating with the external temperature. However, the maintenance of a relatively high body temperature imposes a requirement for precise regulatory mechanisms, since further large elevations of temperature cause nerve malfunction and protein denaturation (Vander *et al*., 1994). Not only does the body maintain internal temperature at around 37°C; it also maintains cell temperatures all over the body at the levels which avoid damage. There are regulating processes which operate within the cells and it is the way in which the whole body regulates all cell temperatures that make up the system of human thermoregulation (Parsons, 2003). Human can tolerate a decline in deep body temperatures of 10 °C but only an increase of 5 °C (Drinkwater, 2007). Some people suffer convulsions at the body temperature of 41°C (106°F) and 43°C is the absolute limit for survival of most people (Vander *et al*., 1994).

It is generally accepted that temperature sensors inside the body are situated in the hypothalamus as well as the medulla, spinal cord and other sites. There are two types of thermal sensor distributed across the skin of the body, so called warm and cold receptors. Signals

from these sensors as well as from core sensors are integrated at the hypothalamus (Figure 6). Core temperatures higher than 42°C are associated with a breakdown of cellular proteins and death (Rhaodes and Pflazer, 2003).

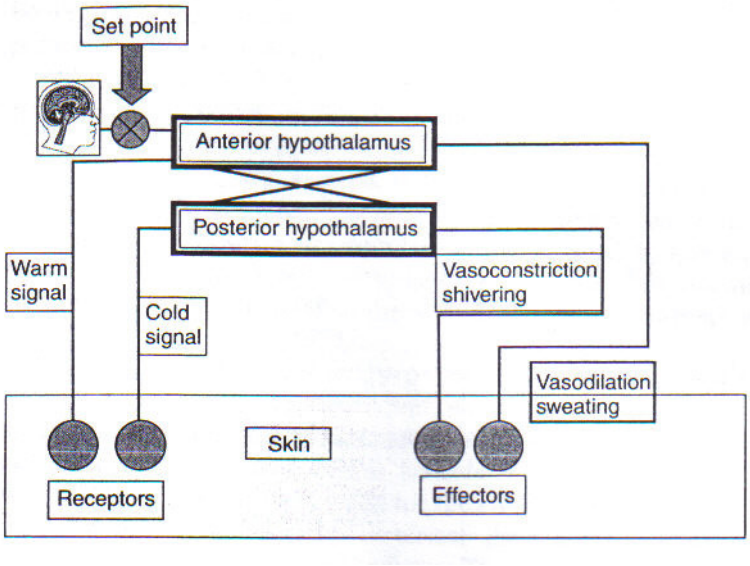


Figure 6. Simplified diagram of the thermoregulatory system (Parsons, 2003).

In the model, if the brain temperature rises above the set-point, the anterior hypothalamus control heat loss (Baron, 2006) causes vasodilation and sweating. Reduction in skin temperature produces vasoconstriction and also contributes to shivering which controlled by posterior hypothalamus (Parsons, 2003).

The thalamus contains the central coordinating center for temperature regulation. This group of specialized neurons at the floor of the brain acts as a thermostat which usually set and carefully regulated at 37±1°C that continually makes thermoregulatory adjustments to deviations from a temperature norm. Unlike the home thermostat, the hypothalamus cannot turn off the heat; it can only initiate responses to protect the body from either a buildup or loss of heat.

Two ways activate the body's heat-regulating mechanism :

1. Thermal receptors in the skin provide input to the central control center.

2. Changes in the temperature of blood that perfuse the hypothalamus directly stimulate this area (Drinkwater, 2007).

Several important generalizations about normal human body temperature should be stressed at the outset :

1. Oral temperature averages about 0.5°C less than rectal, which is generally used.

2. Internal temperature varies several degrees in response to activity pattern and external temperature.

3. There is a characteristic circadian fluctuation so that temperature is lowest during sleep and highest during the awake state even if the person remains relaxed in bed.

4. An added variation in woman is a higher temperature during the last half of the menstrual cycle (Vander *et al*., 1994).

Temperature-sensitive neurons within the body core and found in the hypothalamus, spinal cord, abdominal viscera and great veins. Specific thermoreceptors are integrated with peripheral thermal information at the hypothalamus.

1) Thermosensors

Temperature sensitive receptors have been identified in both the skin and hypothalamus. There is also evidence for thermoregulation in the midbrain, medulla oblongata and spinal cord as well as in blood vessels, the abdominal cavity and a number of other sites.

In the skin, thermoregulators are free nerve endings widely distributed over and within the epidermis. Their signals are carried by either non-myelinated C fibers or small myelinated A fibers and the main afferent spinal pathway is the lateral spinothalamic tract. Thermoreceptors (skin and central) are either warm and cold types, according to the response to stimuli (Persons, 2003). Temperature sensation is encoded by thermoreceptors located on the free endings of small myelinated (Aδ) and unmyelinated (C) fibers. Separate receptors with discrete receptive fields exist for encoding warm and cold sensations (Wang, 1995).

1. Warm fibers are active when the skin temperature between 30-43 °C

2. Cold fibers are active when the skin temperature between 15-38 °C

Vanilloid receptor (TRPV1) are located predominantly on nociceptive afferent fibers and can be activated by capsaicin. Physiologically, this senses noxious heat (> 43°C) (Baron, 2006).

2) Peripheral thermoreceptor regulation of body temperature

Peripheral receptors measure temperature in the skin. These receptors are naked, temperature-sensitive nerve ending and selectivity respond to cold or warm stimuli (Figure 7). While both types of temperature receptors are found throughout the body surface, cold receptors are ten times more numerous. Nerve impulses from peripheral receptors enter the spinal cord and ascend to the brain, to be integrated in the hypothalamus with temperature information from the body core (Rhoades and Pflanzer, 2003).

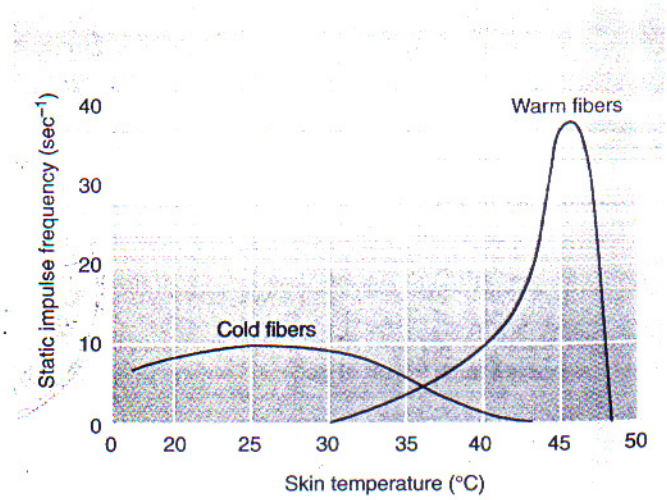


Figure 7. Static discharge frequency of cold and warm nerve fibers as a function of skin temperature (Rhoades and Pflanzer, 2003)**.**

3) Central integration of thermal information

Core and skin temperature are integrated in the hypothalamus. Because the hypothalamus itself is maintained at core temperature, changes in hypothalamic temperature are the single most important input determining thermoregulatory responses. When skin and core

temperature deviate from a regulated value, the hypothalamus initiates a number of physiological responses that modify heat loss or heat gain. These responses include regulation of sympathetic neural control of sweat glands. The hypothalamus also can directly stimulate motor nerves that control skeletal muscle shivering. This pathway modify voluntary skeletal muscle activity through an influence on the cerebral cortex (Rhoades and Pflanzer, 2003) (Figure 8).

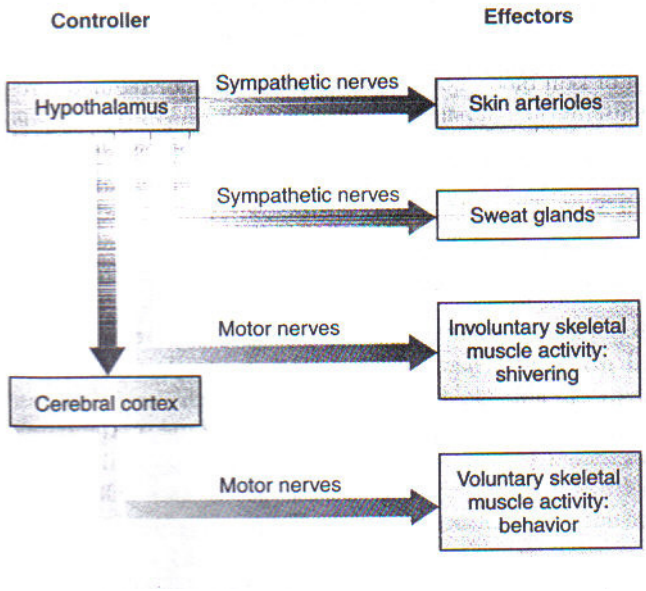


Figure 8. Hypothalamically controlled physiological mechanisms for heat loss or heat gain (Rhoades and Pflanzer, 2003)**.**

Response to heat

1. Vasodilation : Skin vasodilation cause the increase heat loss. During vasodilation venous blood returns near to the skin hence increasing the availability of heat loss from the skin to the environment. Arterio-venous anastomoses deep to the skin capillaries can open and reduce the fall in temperature along the length of the artery, hence increasing arterial temperature, raising skin temperature and increasing heat loss.

2. Sweating : When the body temperature raises, sweat is secreted over the body to allow cooling by evaporation. There are two types of sweat glands :

Apocrine glands, found in the armpits and pubic regions, are generally vestigial, and are responsible for the distributed odour in these regions.

Eccrine glands distributed through out the body (mainly on the forehead, neck, trunk, back of forearm and hand and fewer on thighs soles, and palms). It is the eccrine glands that perform the thermoregulatory function (Table 2).

Table 2. Summary of thermoregulatory effector responses to increases and skin temperature (Rhoades and Pflanzer, 2003).

Response	Mechanism	Effect on heat gain or loss
Skin vasodilation	Decreased sympathetic outflow to skin resistance vessels	Warmer skin increases heat loss to environment; increased convective heat transfer from core to skin reduces core temperature
Sweating	Sympathetic stimulation of sweat glands	Increased evaporation of water from the skin increases heat loss
Behavior	Voluntary skeletal muscle contraction	Shelter from the sun reduces radiant heat gain; reduced clothing increases conduction and convection of heat from the skin

Response to cold

1. Vasoconstriction : To reduce heat loss. Cold vasoconstriction still allows some blood flow for the required small amount of oxygen to reach the cells. In the limbs, a countercurrent heat exchange occurs due to constriction of superficial veins so that cool blood from the skin returns along the vanae comitans close to the artery, hence gaining heat and returning to the body core.

2. Shivering : Both skin temperature and core temperature affect the onset of shivering which can be both voluntary and involuntary. Shivering describes as the simultaneous asynchronous contraction of the muscle fibres in both the flexor and exterior muscle; i.e. activity

producing heat with no net external muscular work. If the body temperature falls then metabolic rate begins to increase, first due to an increase in muscle tone (causing stiffness) and then due to shivering (Parsons, 2003) (Table 3).

Table3. Summary of thermoregulatory effector responses to decreases and skin temperature (Rhoades and Pflanzer, 2003).

Response	Mechanism	Effect on heat gain or loss
Skin vasoconstriction	Increased sympathetic outflow to skin resistance vessels	Cooler skin reduces heat loss to environment; reduces convective heat transfer from core to skin maintains core temperature
Shivering	Involuntary skeletal muscle contraction	Increased energy expenditure increases heat production
Behavior	Voluntary skeletal muscle contraction	Posture changes reduce surface exposed to cold; movement to warmer environment reduces heat loss; increased clothing traps air near the skin, reducing heat loss by convection

Pyrexia or fever

Pyrexia or fever is a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other disease states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive. Normally, the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator such as cytokines like interleukin(IL)-1, interleukin-6, interleukin-11, interferon **(**IFN) (Brahmer and Sande, 2001); interleukin 1β, interleukin α, interleukin β, and TNF-α**,** which increase the synthesis of prostaglandin E_2 (PGE_2) near preoptic hypothalamus area and body temperature. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilates the blood vessels and increases sweating to reduce the

temperature; but when the temperature becomes very low hypothalamus protects the internal temperature by vasoconstriction (Chattopadhyay *et al*., 2005).

Fever is defined as a core body temperature above the normal daily variation. Normal body temperature is 37-38 °C and varies by as much as 0.6 °C throughout the day. The core temperature, measured orally or rectally, is usually lowest in the morning and highest between 4.00 and 6.00 PM.

Several endogenous and exogenous pyrogens can cause fever. Exogenous pyrogens, such as toxin, products of microbes and microbes themselves cause release of endogeneous pyrogens called cytokines. Most of these cytokines are produced by macrophages in the reaction to exogeneous pyrogens. These cytokines cause the hypothalamus to increase prostaglandin synthesis, which is thought to cause an upward shift of the normal core temperature set point (Brahmer and Sande, 2001).

Fever is perhaps the oldest and most universally known hallmark of disease. It occurs not only in mammals but also in birds, reptiles, amphibian and fish. When it occur in homeothermic animals, the thermoreguratory mechanisms behave as if they were adjusted to maintain body temperature at a higher than normal level. The temperature receptors then signal that the actual temperature is below the new set point, and the temperature-raising mechanism are activated. This usually produces chilly sensations due to cutaneous vasoconstriction and occasionally enough shivering to produce a shaking chill. However, the nature of the response depends on the ambient temperature. The temperature rise in experimental animals injected with a pyrogen is due mostly to increased heat production if they are in a cold environment and mostly to decrease heat loss if they are in a warm environment.

The pathogenesis of fever are summarized in Figure 9. Toxin from bacteria such as endotoxin act on monocytes, macrophages, and Kuffer cells to produce cytokines that act as endogeneous pyrogens (EPs). There is good evidence that IL-1β, IL-6, β-IFN, and TNF-α can act independently to produce fever. However, very high temperature is over 41°C (106 °F) for prolonged periods, some permanent brain damage results. When it is over 43°C, heat stroke develops and death is common (Ganong, 1997).

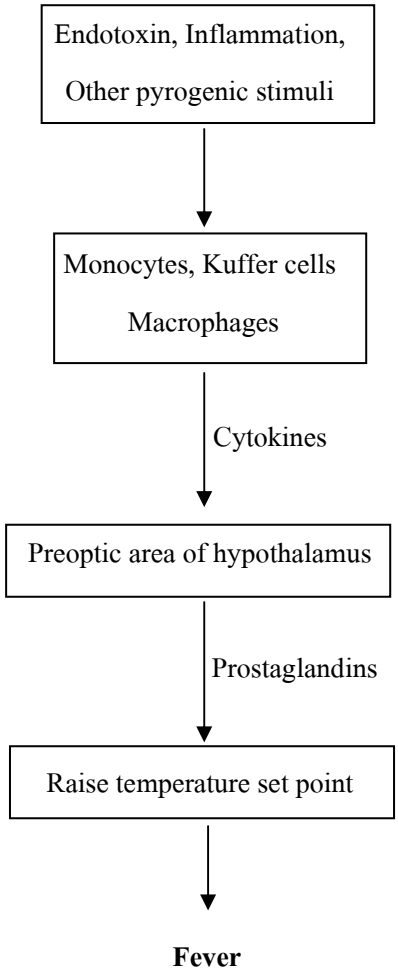


Figure 9. Pathogenesis of fever (Ganong, 1997).

Inflammation

The definition of inflammation is the body's response to tissue injury (Gould, 2002). Inflammation is a defense reaction of the organism and its tissue to injurious stimuli that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be included, maintained or aggravated by many diseases (Gupta *et al*., 2003). The aim is to repair the

damage or at least to limit it and also to remove the cause, for example, bacteria or foreign bodies (Silbernagl and Lang, 2000).

Cause of inflammation

Inflammation is associated with many different types of tissue injury. Causes include direct damage (cuts, sprains), chemical such as acids, ischemia and cell necrosis or infarction, allergic reactions, physical agents (thermal injuries or burns, radiation), foreign bodies (splinters or dirt) and infection (Gould, 2002). Inflammation can be classified based on duration of inflammation as acute and chronic inflammation.

I. Acute Inflammation

Acute inflammation is a rapid response to an injourous agent that serves to deliver mediators of host defense (Kumar *et al*., 2005)**.** This response is relatively non-specific, its main roles being to clear away dead tissues, protect againt local infection and allow the immune system access to the damaged area (Steven and Lowe, 2000) and can be characterized as a cascade of events that result in complex, yet coordinate, interaction between blood leukocytes, blood vessels and cells of the tissue involved. These events are directed toward removal injurious agent and restoration of normal tissue structure and function. However, dysregulated inflammatory processes cause many human diseases (Alex and Peter, 1999). The cardinal signs of acute inflammation are

1. **Redness (rubor):** An acutely inflamed tissue appears red, for example skin affected by sunburn, cellulitis cause by bacterial infection or acute conjunctivitis. This is due to dilatation of small blood vessels within the damaged area.

2. **Heat (calor):** Increase of temperature on the skin is seen only in peripheral parts of the body. It is due to increased blood flow (hyperaemia) through the region, resulting in vascular dilatation and the delivery of warm blood to the area.

3. **Swelling (tumor):** Swelling results from edema, the accumulation of fluid in the extra vascular space as part of the fluid exudate, the physical mass of the inflammatory cells migrating into the area.

4. **Pain (dolor):** For the patient, pain is one of the best known features of acute inflammation. It results partly from the stretching and distortion of tissues due to inflammatory edema and, in particular, from pus under pressure in an abscess cavity. Some of the chemical mediators of acute inflammation, including bradykinin, prostaglandins and serotonin, are known to induce pain.

5. **Loss of function:** Loss of function is a well-known consequence of inflammation. Movement of an inflamed area is consciously and reflexly inhibited by pain, while severe swelling may physically immobilise the tissues (Macfarlane *et al*., 2000). These in turn give rise so called fifth sign of inflammation (Norris, 2004)

Many factors which mediate the events of acute inflammation have been documented. These chemical mediators of inflammation are important, since the process can be modified by drug therapy to minimize unwanted and potentially damaging effects. The mediators either come from cells or are plasma-derived (Table 4). Plasma derived mediators gain entry to the damage area via the inflammatory exudates. They are mostly precursor proteins, which are activated by proteolytic enzymes and, once activated, generally have short half-lives. Once in tissues, they are rapidly inactivated by a variety of enzymatic or scavenging systems.

Histamine is the main pre-formed mediator of inflammation. Released from mast cells, basophils and platelets, it causes transient dilatation of arterioles, increases permeability in venules, and is the primary cause of increased vascular permeability in the first hour after injury.

Both prostaglandins and leukotrienes are derived by local synthesis by arachidonic acid. This long-chain fatty acid is liberated from cell membranes by activation of the enzyme, phospholipase A_2. There are two main pathways in arachidonic acid metabolism :

1. The cyclo-oxygenase pathway produces : thromboxane A_2 (TXA_2), which aggregates platelets and causes vascular constriction; prostacyclin (PGI_2), which inhibits platelet aggregation and dilates vessels; and stable prostaglandins (PGE_2, $PGF_{2\alpha}$, PGD_2), which cause vasodilation and increase vascular permeability. PGE2 also cause pain. There are two forms of cyclo-oxygenase termed COX-1 and COX-2. COX-1 is normally present in cells as a

constitutively expressed enzyme, whereas COX-2 is specially induced in cells where it plays a role in inflammation.

2. The lipoxygenase pathway produces leukotrienes (LTC_4, LTD_4, LTE_4), which cause vasoconstriction and increase permeability in vanules. Leukotriene (LTB_4) stimulates leukocyte adhesion to endothelium.

Platelet activating factor (PAF) is synthesized by mast cells or basophils and can be stimulated by IgE-mediated release. Also synthesized by platelets, neutrophils, monocytes, and endothelium, it is a specialized phospholipids compound, which causes vasoconstriction, increased vascular permeability, and platelet aggregation, and is at least a thousand times more potent than histamine. It also stimulates the synthesis of arachidonic acid metabolites.

Cytokines are polypeptide products of activated lymphocytes and monocytes. The main cytokines participating in acute inflammation are interleukin-1 (IL-1) interleukin-8 (IL-8) and tumour necrosis factor alpha (TNFα). These are responsible for :

- Induction of cell adhesion molecules on endothelium.
- Induction of PGI_2 (prostacyclin) synthesis.
- Induction of PGF synthesis.
- Fever, anorexia, and stimulation of acute-phase protein synthesis by liver.
- Stimulation of fibroblast proliferation and secretory activity.
- Attraction of neutrophils into damaged area (IL-8).

The chemokines are a family of factors secreted by leukocytes and endothelial cells in response to tissue damage and other inflammatory mediators. They are locally bound to extracellular metrix and heparin-sulphate proteoglaycans of cells and establish a concentration gradient away from the focus of inflammation. Neutrophil rolling causes neutrophils to encounter chemokines bound to proteoglycans on endothelial cells. Specific chemokine receptor are activated and this signals for activation of leukocyte integrins, mediating adhesion and emigration. Chemokines are removed from the circulation by the duffy antigen receptor for chemokines (DARC) expressed on red cells.

Nitric oxide is a small molecule that is locally synthesized by endothelium and macrophages through the activity of the enzyme, nitric oxide synthase. It is a powerful cause of

vascular dilation, and increase vascular permeability. As an important inactive oxygen intermediary, it can also mediate cell and bacterial killing.

The complement system comprises a set of plasma proteins with important roles in immunity and inflammation. There is a cascade of activation, with production of numerous intermediary activated peptides. The main products with roles in acute inflammation are as follows:

- C3a increases vascular permeability by liberating histamine from mast cells or platelets
- C5a increases vascular permeability by liberating histamine from mast cells or platelets, is chemotactic to neutrophils and induces endothelial cell adhesion molecules.
- C345 is chemotactic to neutrophils.
- C3b opsonizes bacteria and facilitates neutrophil phagocytosis.

The kinin are small peptides derived from plasma precursors by proteolytic cleavage. The system is activated by one of the coagulation proteins, activated Hageman factor (Factor XII); this cleaves the peptide prekallikrein to kallikrein. Kallikrein stimulates a high molecular weight kininogen to form bradykinin a powerful mediator of increased vascular permeability, causes pain, vasodilatation, edema (Vane and Botting, 1987) and activates the complement system.

The clotting pathway is responsible for coagulation of blood by formation of fibrin from fibrinogen. Factor XII (Hagman factor) is activated the inflammatory exudates when it comes into contact with collagen outside the vessel. It then stimulates deposition of fibrin, fibrinopeptides are formed. These cause increased vascular permeability, as well as being chemotactic for neutrophils.

The thrombolytic pathway. The enzyme plasmin (generated by plasminogen activator derived from endothelium by the action of bradykinin) is a proteolytic enzyme with several roles in inflammation.

In acute inflammation, these factors act in concert to bring about the structural and functional changes (Steven and Lowe, 2000).

Table 4. Main groups of mediators involed in acute inflammation (Steven and Lowe, 2000)

Cellular mediators of acute inflammation	
Stored	**Active synthesis**
Histamine	Prostaglandins
	Leukotrienes
	Platelet activating factor
	Cytokines
	Nitric oxide
	Chemokines
Plasma-derived mediators of acute inflammation	
Kinin system	Bradykinin
Clotting pathway	Activated Hageman factor
Thrombolytic system	Plasmin
Complement pathway	C3a, C3b, C5a

Pathophysiology of acute inflammation

The inflammation process is basically the same regardless of the cause. The severity of the inflammation may very with the specific situation. Tissue injury damages cells. Mast cells and platelets release chemical mediators such as histamine , serotonin, prostaglandins and leukotrienes into the interstitial fluid and blood. These chemicals affect blood vessels and nerves in the area. Note that many anti-inflammatory drugs and antihistamines reduce the effects of some of these chemical mediators.

Although nerve reflexs at the site of injury cause immediate transient vasoconstriction, the rapid release of chemical mediators results in local vasodilation, which increases blood flow in the area (hyperemia). Capillary membrane permeability also increases, allowing plasma proteins to shift into the interstitial space along with more fluid. The increased fluid dilutes any toxic material at the site, while the globulins serve as antibodies, and fibrinogen

forms a fibrin mesh around the area in an attempt to localize the injurious agent. Vasodilation and increase capillary permeability make up the vascular response to injury.

During the cellular response leukocytes are attracted (chemotaxis) to the area of inflammation as damaged cell release their contents. The chemicals at the site of injury act like magnets for cells. First neutrophils (polymorphonuclear leukocytes, PMNs) and later monocytes and macrophages collect along the capillary wall (marginate) and then move through the wider separations in the wall into the interstitial area. There they destroy and remove foreign material, microorganisms and cell debris by phagocytosis, thus preparing the site for healing. When phagocytic cells die at the site, lysosomal enzymes are released that damage the nearby cells and prolong inflammation. If an immune response or blood clotting occurs, these process also enhance the inflammatory response.

As excessive fluid and protein collects in the interstitial compartment, blood flow in the area decreases, and fluid shifts out of the capillary are reduced. Severely reduced blood flow can decrease the nutrients available to the undamaged cells in the area and prevent the removal of wastes. This may cause additional damage to the tissue (Figure 10) (Gould, 2002).

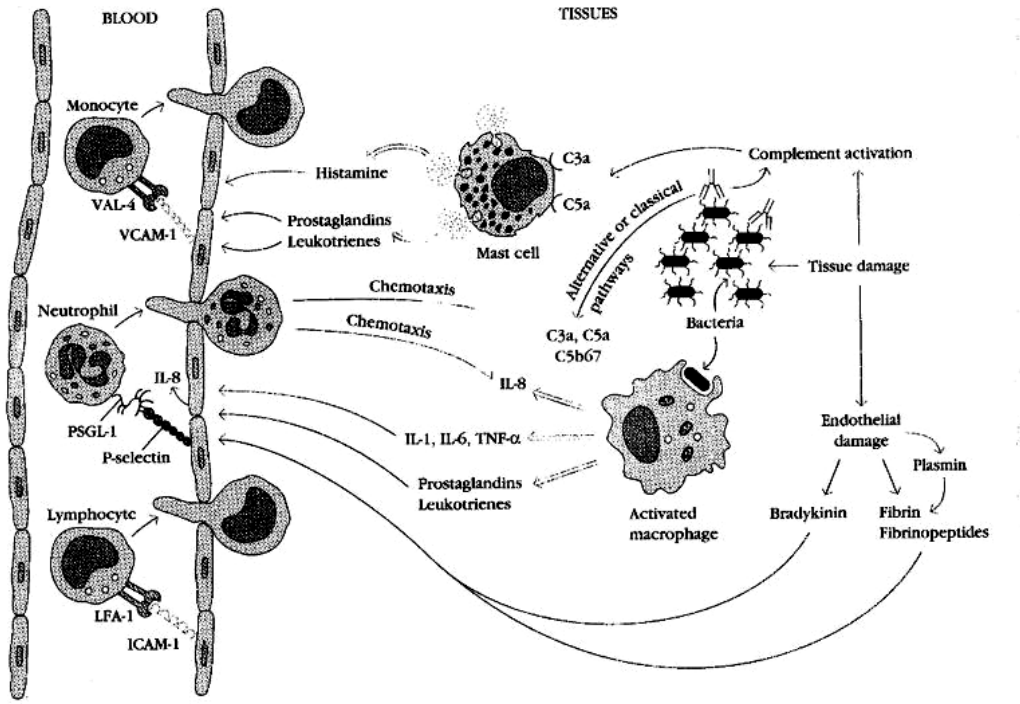


Figure 10. The cells and mediators involved in a local acute inflammatory response (Gould, 2002).

II. Chronic Inflammation

If the damaging stimulus persists, the processes of continuing tissue necrosis, organization and repair all occur concurrently. In addition to acute inflammation, the specific defences of the immune system are activated around the area of damage and tissue are infiltrated by activated lymphoid cells. Histological examination of an effected area will show necrosis cell debris, acute inflammatory exudates, vascular and fibrous granulation tissue, lymphoid cells, macrophages and collageneous scar. This state termed chronic inflammation, will persist until the damaging stimulus is removed or neutralized (Steven and Lowe, 2000).

Chronic inflammation may develop following an acute episode of inflammation when the cause is not completely eradicated, or it may develop insidiously owing to chronic

irritation, specific bacteria or long-term abnormal immune responses (Gould, 2002) and suppresses apoptosis. Increases cell division and decreased apoptosis lead to survival and expansion of a mutated cell population (Murphy and Ward, 2005).

Pathophysiology of chronic inflammation

Characteristics of chronic inflammation include less swelling but the presence of more lymphocytes, and fibroblasts than in acute inflammation and macrophages have been unable to completely clear (debride) the area of foreign substances. This material may be dead cells, extracellular blood, or sand or dirt in some cases. Either way, the material is surrounded by collagen to isolate it from the body. This mass of encapsulating scar is called a granuloma (Norris, 2004). More collagen is produced in the area resulting in more fibrous scar tissue forming. Granulomas may develop when an area is walled off by fibrous tissue but the cause has not been removed, as in tuberculosis. Frequently, more tissue destruction occurs with chronic inflammation (Gould, 2002).

Aspirin (acetylsalicylic acid)

Aspirin (Figure 11) is the longest-standing NSAID and is an effective analgesic. Aspirin remains the drug of choice for many sorts of mild to moderate pain and certain types of severe pain. It is not effective in the treatment of visceral pain (e.g. myocardial infraction, renal colic, acute abdomen) (Neal, 1992).

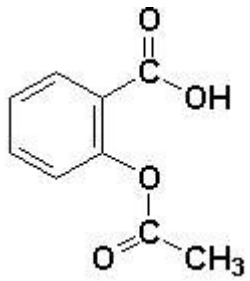


Figure 11. Structure of aspirin

1. Pharmacokinetics

Aspirin is absorbed rapidly in the stomach and upper intestine. Peak plasma levels occur 30 to 40 minutes after aspirin ingestion (Caterina and Patrono, 2002). Gastrointestinal absorption depends on the gastric pH on the release characteristics of the tablet formulation and inhibition of platelet function is evident by 1 hour. A variable hepatic first-pass metabolism is recognized (Reid *et al*., 1992) In contrast, it can take up to 3 to 4 hours to reach peak plasma level after administration of enteric-coated aspirin. The oral bioavailability of regular aspirin tablets is approximately 40 to 50 % over a wide range of doses. A considerably lower bioavailability has been reported for enteric-coated tablet and sustained-release, microencapsulated preparations. The plasma concentration of aspirin decays with a half-life of 15 to 20 minutes. Despite the rapid clearance of aspirin from the circulation, the platelet-inhibitory effect lasts for the life span of the platelet because aspirin irreversibly inactivates platelet COX-1. The mean life span of human platelets is approximately 10 days. Therefore, approximately 10% of circulating platelets is

replaced every 24 hours and 5 to 6 days following aspirin ingestion, approximately 50% of platelets function normally. Aspirin is metabolized predominantly in the liver by the cytochrome P-450 system, most commonly the CYP 2C9 isoform, and excreted in the urine. This metabolism must be taken into consideration when prescribing aspirin for patients with hepatic or renal dysfunction (Simon, 2003).

2. Pharmacodynamics

The anti-inflammatory activity of the NSAIDs is similar in mechanism to that the aspirin and is mediated chiefly through inhibition of biosynthesis of prostaglandins. Inflammation is reduce by decreasing the release of the mediators from granulocytes, and reverse vasodilatation. To varying degrees, all NSAIDs are inhibitors of prothrombin synthesis; all are analgesic, antipyretic, anti-inflammatory and all inhibit platelet aggregation. They are all gastric irritants as well (Donald and Payan, 1992).

2.1 Mechanism of action

Aspirin acetylates prostaglandin(PG) H-synthase binds covalently with a serine residue of the enzyme (Neal, 1992) causing irreversibly inhibits its cyclooxygenase (COX) activity and potent inhibitory effects on platelet adhesion and aggregation (Reid *et al*., 1992). This enzyme catalyzes the first committed step in prostanoid biosynthesis, the conversion of arachidonic acid to PGH_2. There are two isoforms of PGH-synthase (PGHS) referred to as PGHS-1 and PGHS-2 or COX-1 and COX-2.

COX-1 or prostaglandin synthase H_1, which typically regulates normal cellular processes, is stimulated by hormones or growth factors. It is constitutively expressed in most tissues and is inhibited by all NSAIDs. COX-1 is important in maintaining the integrity of the gastric and duodenal mucosa, and many toxic effects of NSAIDs on the gastrointestinal tract are attributed to its inhibition. The other isoform, COX-2 or prostaglandin H_2 is an inducible enzyme and is usually undetectable in most tissues. Its expression is decreased during state of inflammation or experimentally in responses to mitogenic stimuli. This isoform constitutively

expressed in the brain (Simon, 2003), undetectable in most mammalian tissues. Aspirin is a more potent inhibitor of platelet COX-1 than COX-2. Human platelet and vascular endothelial cells process PGH_2 to produce thromboxane A_2 (TXA_2) and prostacyclin (PGI_2), respectively. TXA_2 induces platelet aggregation and vasoconstriction, whereas PGI_2 inhibits platelet aggregation and induces vasodilation. Vascular PGI_2 production appears to be largely dependent on COX-2 activity of endothelial cells (Caterina and Patrono, 2002). The activity of COX-1 and COX-2 is inhibited by all of the presently available NSAIDs to a greater or lesser degree. The clinical effectiveness of these drugs is believed to be due to the effects on COX-2, whereas many of the mechanism-based toxic effects are thought to be secondary to inhibition of COX-1 (Simon, 2003).

2.2 Analgesic effects

The analgesic action of aspirin is exerted both peripherally and centrally, but the peripheral action predominate. It's the analgesic action associated with its anti-inflammatory action and results from the inhibition of prostaglandin synthesis in the inflamed tissue. Prostaglandins produce mild pain by themselves, but potentiate the pain caused by other mediators of inflammation (e.g. histamine, bradykinin) (Neal, 1992). Aspirin is most effective in reducing pain of mild to moderate intensity. It alleviates pain of varying causes, such as that of muscular, vascular and dental origin, postpartum states, arthritis, and bursitis. Aspirin acts peripherally through its effect on inflammation but probably also depresses pain stimuli at a subcortical site (Donald and Payan, 1992).

2.3 Antipyretic effects

Aspirin reduces elevated temperature, whereas normal body temperature is only slightly affected. The fall in temperature is related to increased dissipation of heat caused by vasodilation of superficial blood vessels. The antipyretic may be accompanied by profuse sweating. The fever associated with infection is thought to result from two actions. The first is the production of prostaglandins in the central nervous system in response to bacterial pyrogens. The second is the effect of interleukin-1 on the hypothalamus. Interleukin-1 is produced by

macrophages and is released during inflammatory responses, when its principal role is to activate lymphocytes. Aspirin blocks both the pyrogen-induced production of prostaglandin and the central nervous system response to interleukin-1 and so may reset the “temperature control” in the hypothalamus, thereby facilitating heat dissipation by vasodilatation (Donald and Payan, 1992).

2.4 Anti-inflammatory effects

The role of prostaglandins in inflammation is to produce vasodilatation and increased vascular permeability (Neal, 1992). In addition to reducing the synthesis of eicosanoid mediators of inflammation, aspirin also interferes with the chemical mediators of the kallikrein system. As a result, aspirin inhibits granulocyte adherence to damaged vasculature, stabilizes lysosomes, and inhibits the migration of polymorphonuclear leukocytes and macrophages into the site of inflammation (Donald and Payan, 1992).

2.5 Platelet effects

Aspirin affects hemostasis. Single doses of aspirin produce a slightly prolonged bleeding time, which doubles if administration is continued for a week. The change is explained by the inhibition of platelet aggregation secondary to inhibition of thromboxane synthesis. Because thromboxane accelerates platelet aggregation, aspirin inhibits platelet aggregation for up to 8 days until platelets are formed. If potential bleeding complications are a concern in association with surgery, aspirin should be stopped 1 week prior to the operating (Donald and Payan, 1992).

3. Adverse effects

3.1 Gastrointestinal tract

At the usual dosage, the main adverse effect is gastric upset (intolerance). This effect can be minimized with suitable buffering (taking aspirin with meals followed by a glass of water or antacid). The gastritis that occurs with aspirin may be due to irritation of the gastric mucosa by the undissolved tablet or to inhibition of protective prostaglandins (Katzung and Furst, 1998). Damage to the mucosa of the gastrointestinal tract seem to be mainly a consequence of prostaglandin synthesis inhibition, rather than a directly erosive action of drugs. Prostaglandins (PGE_2 and PGI_2) inhibit gastric acid secretion, increase blood flow through the gastric mucosa and have a cytoprotective action PGE_2 and some analogues induce healing in peptic ulcer. By inhibiting prostagllandin formation, NSAIDs may cause ulceration by producing mucosal ischemia and by impairing the protective mucus barrier, thus exposing the mucosa to the damaging effect of acid (Neal, 1992).

3.2 Liver

Aspirin administered regularly in doses greater than 50 mg/kg can produce mild and reversible hepatic damage. This is usually manifested as an increase in aminotransferase values but biopsies reveal focal hepatocellular necrosis, hepatocytic swelling, intracellular and extracellular acidophilic bodies and portal inflammation. A small number of patient experience more severe hepatic damage with jaundice, prolonged prothrombin time with bleeding or intravascular coagulation. Aspirin also may precipitate hepatic encephalopathy in patients with chronic liver disease. The hepatotoxicity generally occurs only after several months of treatment and appears as cholestatic jaundice with markedly elevated values in hepatic function tests and histologic evidence of necrosis, portal infiltrates and cholestasis (Foegh and Ramwell, 2001).

3.3 Pregnancy and lactation

Aspirin has been examined extensively for their potential adverse effects on the pregnant woman, the fetus and on the nursing neonate whose mother is receiving one of these

drugs. It is presumed that, like aspirin, the other NSAIDs prolong gestation and labor, increase maternal blood loss during delivery and may cause fetal intracranial hemorrhage. Fetal growth retardation may be related to inhibition of glucose-induced insulin release. No teratogenic effects have been substiated. Large dose of aspirin in the mother can induce bleeding or rash in nursing infant (Foegh and Ramwell, 2001).

3.4 Central nervous system effects

With higher doses, patients may experience decreased hearing and vertigo which are reversible by reducing the dosage. Still larger doses of salicylates cause hyperpnea through a direct effect on the medulla. At low toxic salicylate levels, respiratory alkalosis may occur as a result of the increased ventilation. Later, Acidosis supervenes from accumulation of salicylic acid derivatives and depression of the respiratory center (Katzung and Furst, 1998).

4. Drug interaction

Drugs that enhance salicylate intoxication include acetazolamide and ammonium chloride. Alcohol increases gastrointestinal bleeding produced by salicylates. Aspirin displaces a number of drugs from protein binding sites in the blood. These include tolbutamide, chlorpropamide, NSAIDs, methotrexate, phenytoin and probenecid. Corticosteroids may decrease salicylate concentration. Aspirin reduces the pharmacologic activity of spironolactone, competes with penicillin G for renal tubular secretion, and inhibits the uricosuric effect of sulfinpyrazone and probenecid (Donald and Payan, 1992).

Morphine

Morphine (Figure 12) is the prototype opioid agonist to which all other opioids are compared. In human, morphine produces analgesia, euphoria, sedation and a diminished ability to concentrate. Other sensations include nausea, a felling the body warmth, heaviness of the extremities, dryness of the mouth and especially in the cutaneous areas around the nose. The cause of pain persists, but even low doses of morphine increase the threshold to pain and modify the perception of noxious stimulation so that it is no longer experienced as pain. In the absence of pain, however, morphine may produce dysphoria rather than euphoria (Stoelting and Hillier, 2006).

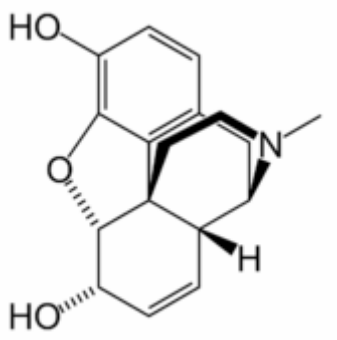


Figure 12. Structure of morphine

1. Pharmacokinetics

Following oral administration, morphine is readily absorbed from the upper part of the intestines. Opioids are absorbed readily after subcutaneous or intramuscular injection (Gutstein and Akill, 2001). However, the bioavailability of oral morphine is low (35%) due to a large “first pass” metabolism in the liver. Morphine is metabolized to morphine-3-glucuronide and morphine-6-glucuronide by conjugation with glucuronic acid (Bracht, 2000) in hepatic and extrahepatic sites, especially the kidneys. About 75% to 85% of a dose of morphine appears as

morphine-3-glucuronide and 5% to 10% as morphine-6-glucuronide (a ratio of 9:1) (Stoelting and Hillier, 2006). While morphine-6-glucuronide is an active metabolite with profound analgesic activity (Bracht, 2000) and depression of ventilation via its agonist action at μ receptors. Renal metabolism makes a significant contribution to the total metabolism of morphine, morphine-3-glucuronide is pharmacologically inactive (Stoelting and Hillier, 2006). Morphine is high water and low fat solubility, the onset of analgesia is slow and the duration correspondingly long (8-10 h) (Bracht, 2000).

2. Pharmacodynamics

2.1 Mechanism of action

Opioid receptors are found in the central nervous system and gastrointestinal tract and to a lesser degree, in peripheral tissues. Opioid drugs manifest analgesic effects primarily by binding to and activating (agonizing) opioid receptor in the nervous system (CNS). The interaction of exogenous opioids, for example, morphine and opioid receptors, mimics the interaction seen when endogenous opioid peptides (dynorphins, endorphins, enkephalins) bind with these same receptors

The three generally recognized classes of opioid receptors are the mu (μ), delta (δ) and kappa (κ) receptors and epsilon (ε) receptors were formerly classified as opioid receptors because opioids can bind to them (Lipman and Jackson, 2004).

Mu-opioid receptor : Morphine acts mainly on the μ-receptor, which is primarily responsible for the analgesic actions. Most of the common opioids are full agonists at the μ-receptor. This receptor is also responsible for the often unwanted effects of opioids, such as respiratory depression, constriction of pupils (miosis), sedation and reduction in motility of the gastrointestinal system, as well as euphoric effects.

Delta-opioid receptor : This opioid receptor occurs at different locations within the brain to the μ-receptor but also mediates analgesia, respiratory depression, euphoria and dependence. The functional significance of this receptor is less clear but seem to be involved more with peripheral pain control. Etorphine is the best agonist.

Kappa-opioid receptor : This receptor occurs in the spinal cord and mediates spinal analgesia and sedation and support only low physical dependence. Pentazocine and etorphine are the best opioid drugs of this site (Table 5) (Drummer, 2001).

Table 5. Receptor selectivities for some opioids (Drummer, 2001).

Opioid	mu (μ)	Delta (δ)	kappa (κ)
Full agonists			
Codeine	+	+	-
Etorphine	+++	+++	+++
Fentanyl	+++	+	-
Methadone	+++	+	+
Morphine	+++	+	+
Pethidine	+++	+	+
Sulfentanil	+++	+	-
Partial agonist			
Buprenorphine	+++	-	Antagonist
Pentazocine	Antagonist	+	++
Antagonist			
Naloxone	Antagonist	Antagonist	Antagonist
Naltrexone	Antagonist	Antagonist	Antagonist

Opioid receptors are composed of glycoproteins found in cellular membranes. These receptors are coupled to G-proteins that modulate potassium and calcium ion conduction (Figure 13). When opioid agonists occupy either μ or δ receptors, they open the potassium ion channel that permits an increase in potassium conductance. The hyperpolarlization inhibits neuronal activity. In contrast, κ receptor activation inhibits calcium entry via a calcium ion channel. Activation of the opioid receptors decreases transmission of signals from the primary

peripheral afferent nerves to higher CNS centers, as well as the processing of the pain stimulus (Lipman and Jackson, 2004).

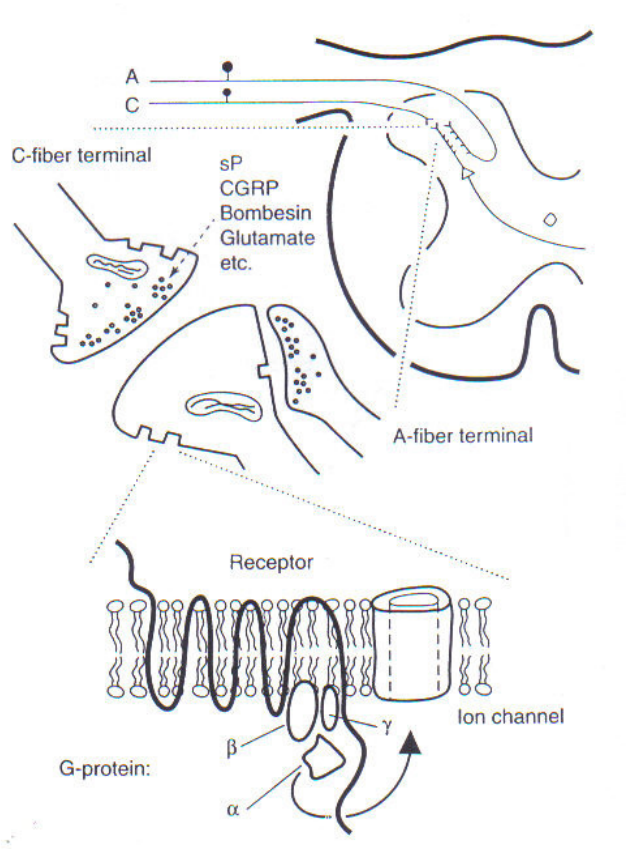


Figure 13. Illustration of synapse Aδ and C fibers with second-order neurons in the dorsal horn of the spinal cord and proposed opioid receptor demonstrating the G protein subunits and close approximation to an ion channel (Lipman and Jackson, 2004).

Activation of the opioid receptors lead to analgesia as well as adverse effects. All three opioid receptor types have know subtypes (Table 5). Two μ subtypes have been best elucidated. Activation of μ_1 leads to supraspinal analgesia, whereas μ_2 activation is commonly thought to be responsible for the adverse sequelae of opioid administration. Activation of κ and δ receptors lead to spinal analgesia, However, κ_3 receptors are thought to mediate supraspinal

analgesia. Activation of δ receptors may actually potentiate μ-receptor-induced analgesia. Clinical implications of the δ- and κ-receptor subtypes have not been fully developed at this time (Lipman and Jackson, 2004).

Table 5. Opioid receptors, subtypes and physiological effect (Lipman and Jackson, 2004).

Receptor	Subtypes	Effects
Mu	Mu 1	Supraspinal analgesia
	Mu 2	Physical dependence
		Euphoria
		Sedation
		Respiratory depression
		Constipation
		Orthostatic hypertension
		Arteriolar/venous vessel dilation
Delta	Delta 1, 2	Spinal analgesia
		Euphoria
		Potentiates mu receptor analgesia
Kappa	Kappa 1, 2, 3	Spinal analgesia
		Sedation
		Miosis
		Supraspinal analgesia (κ_3)

3. Adverse effects

3.1 Cardiovascular system

Morphine can also evoke decreases in systemic blood pressure due to drug-induced bradycardia or histamine release. The administration of morphine in the preoperative medication or before the induction of anesthesia tends to slow heart rate during exposure to volatile anesthetics, with or without surgical stimulation.

3.2 Ventilation

All opioid agonists produce a dose–dependent and gender specific depression of ventilation, primarily through an agonist effect at μ_2 receptors, which lead to a direct depressant effect on brainstem ventilation centers.

3.3 Sedation

The postoperative titration morphine frequently induces a sedation that precedes the onset of analgesia

3.4 Gastrointestinal tract

Commonly used opioids can produce spasm of the gastrointestinal smooth muscles, resulting in a variety of side effects including constipation, and delayed gastric emptying.

3.5 Nausea and vomiting

Nausea and vomiting induced by opioid reflects their direct stimulation of the chemoreceptor trigger zone in the floor of the fourth ventricle.

3.6 Cutaneous changes

Morphine causes the cutaneous blood vessels of the face, neck and upper chest to dilate.

4. Drug interaction

The ventilatory depressant effects of some opioids may be exaggerated by amphetamines, phenothiazines, mono-amine oxidase inhibitors and tricyclic anti-depressant (Stoelting and Hillier, 2006).

Naloxone

The pure opioid antagonist drugs naloxone is morphine derivative with bulkier substituents at the N_{17} position. These agent have a relatively high affinity for μ opioid binding sites and have lower affinity for the other receptors but can also reverse agonists at δ and κ sites.

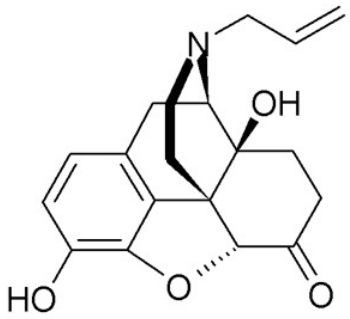


Figure 14. Structure of naloxone

1. Pharmacokinetics

Naloxone have poor efficacy when given by the oral route and a short duration of action (1-2 hours) when given by injection. Metabolic disposition is chiefly by glucuronide conjugation, like that of the agonist opioids with free hydroxyl groups (Way *et al*., 1998). Naloxone appear to be devoid of agonistic actions and probably interact with all types of opioid receptors and apparently acts antagonize the action of endogenous opioids that mobilized by pain or stress and that are involved in the regulation of blood pressure by the CNS, opioid antagonists can reduce the extent of injury in some animal models, perhaps by blocking κ receptors.

Naloxone is almost completely metabolized by the liver before reaching the systemic circulation and thus must be administered parenterally. The drug is absorbed rapidly from parenteral sites of injection and is metabolized in the liver, primarily by conjugation with glucuronic acid, other metabolites are produced in small amounts.

Subcutaneous dose of naloxone produce no discernible subjective effects in human beings and 24 mg causes only slight drowsiness. Small doses (0.4 to 0.8 mg) of naloxone

given intramuscularly or intravenously prevent or promptly reverse the effect of μ receptor agonists. In patients with respiratory depression, an increase in respiratory rate in seen within 1 or 2 minutes. Sedative effects are reversed, and blood pressure, if depressed, returns to normal. Higher dose of naloxone are required to antagonize the respiratory-depressant effects (Gutstein and Akil, 2001).

2. Pharmacodynamics

When given in the absence of an agonist drug, these antagonists are almost inert at dose that produce marked antagonism of agonist effects (Way *et al*., 1998). When given to a morphine-treated subject, the antagonist will completely and dramatically reverse the opioid effects within 1-3 minutes. The patients who are acutely depressed by overdose of an opioid, the antagonist will effectively normalize respiration, level of consciousness, pupil size and bowel activity (Way *et al*., 1992).

3. Adverse effect

Major side effect derive directly from its mechanism of action-antagonism of opioid agonists at all of known opioid receptors. This agent was available for use as an antagonist. It was mixed agonist-antagonist, such that if given after opioid, the opioid effects were reversed. However, when given alone, it produced respiratory depression, cough suppression, miosis and analgesia (Smith, 1995).