CHAPTER II

REVIEW LITERATURE

Physiological Concept of Pain Mechanism

Peripheral Mechanism

Sensory experience begins with the activation of sensory receptors and their primary afferent neurons. According to Johannes Müller's doctrine of Specific Nerve Energies, different sensations are produced following the activation of different specific types of sensory receptors (Müller 1840-2) and the remainder of their sensory channels. For example, stimulation of the sensory retina results in a visual experience, whether the stimulus is light or mechanical deformation of the eye. The sensory receptors that are responsible for detecting stimuli that damage or threaten to damage tissue are known as nociceptors (Sherrington 1906). Sherrington proposed the existence of nociceptors long before they were actually demonstrated in animals (Iggo 1959; Iriuchijima and Zotterman 1960; Bessou and Perl 1969) and in humans (Torebjörk 1974)

Activation of nociceptors results in pain, whether the nociceptors are stimulated by natural forms of noxious stimuli, including strong mechanical, thermal and chemical stimuli (Meyer and Campbell 1981; Van Hees and Gybles 1981; LaMotte et al. 1991) or by electrical stimulation of their axons (Torebjörk and Hallin 1973; Ochao and Torebjörk 1989).

The process by which sensory receptors are activated is called sensory transduction. The events involved in transduction in nociceptors

are complex and include alterations in the environment around nociceptive terminals and responses of these terminals to mechanical (mechanical nociceptors), mechanical and thermal (mechano-thermal receptors) or mechanical, thermal and chemical energy (polymodal nociceptors) (Belmote 1996). Different classes of nociceptors may respond to just one of these forms of energy or they may be activated by two or more. For example, polymodal nociceptors respond to all three. Transduction of mechanical forces is likely to depend on the opening of mechanically sensitive membrane channels either by tension applied directly to the membrane or by activation of second messenger systems (Belmote 1996). Chemical substances may have a non-specification on the membranes of nociceptors or they may interact with specific membrane receptor molecules. Many irritant substances have a non-specific action that depolarizes nociceptors terminals. An example of a membrane receptor-mediated event is the opening of ligand-gated cation channels in response to binding of 5-hydroxytryptamine (5-HT; serotonin) to 5-HT_3 receptors on nociceptors. Another example is the activation of vanilloid receptors (VR1 receptors) by capsaicin. However, VR1 receptors are also activated by heat, and so these receptors may play a major role in thermal nociception; in addition, the responses of VR1 receptors are modulated by pH, and so they may be involved in the increase in pain that occurs as tissue pH is lowered (Tominaga et al. 1998). A third mechanism that may contribute to nociceptive transduction is the activation of G-protein coupled membrane receptors which initiate a cascade of second messenger events that may alter the excitability of nociceptors terminals, for example by phosphorylation of ion channels. Second and third messenger cascades are also involved in longer term events in which nociceptors undergo plastic changes in their properties.

The initiation of nerve impulses in nociceptors is in response to the development of a receptor (or generator) potential, a depolarization that may exceed threshold for the generation of nerve impulses (Belmotte 1996). The ability of the receptor potential to trigger nerve impulses can be affected not only by the transduction events already mentioned, but also by changes in extracellular potassium concentration, the activity of the Na^+-K^+ pump, and alterations in voltage-gated Na^+, K^+ and Ca^{2+} channels, For instance, the discharges of nociceptors can be prevented by administration of local anesthetics or, in experimental preparations, of tetrodotoxin, which block voltage-gated Na^+ channels. However, tetrodotoxin may be in-effective if nociceptors contain tetrodotoxin-resistant sodium channels (Gold et al. 1996).

Primary afferent nociceptors have either small myelinated Aδ or unmyelinated C axons. By contrast, sensitive cutaneous mechanoreceptors are often supplied by large myelinated Aβ fibers, and muscle stretch receptors are innervated by largest myelinated group I fibers (Willis and Coggeshall 1991). Activation of cutaneous Aδ nociceptors results in a sensation of pricking or sharp pain, whereas activation of cutaneous C nociceptors caused burning or dull pain. These are also term "fist" and "second" pain, since pricking pain occurs at a shorter latency after a stimulus than does burning pain (Lewis 1942; Treede et al.1995). On the other hand, activation of the nociceptors that supply muscle or other deep somatic structures, such as joints, fascia and bone, results in aching or cramping pain (Lewis 1942; Mense 1993; Schible and Grubb 1993). Nociceptors are also found in viscera (Ness and Gebhart 1990; Cervero 1994). These are more difficult to study in humans than are cutaneous nociceptors. However, their properties have been delineated in experimental studies.

Although many nociceptors respond to strong mechanical stimuli, some do not. These have been termed "silent" nociceptors (Schaible and Schidt 1985; Schmidt et al. 1994). Silent nociceptors can undergo a process of peripheral sensitization. This process results in their becoming highly responsive to even weak mechanical stimuli. Peripheral sensitization is often associated with tissue injury and inflammation and results from exposure of nociceptors endings to inflammatory agents, such as prostaglandins, bradykinin, histamine, ATP, cytokines and 5-hydroxytryptamine. Inflammatory agents can be released by neurons, mast cell and platelets, or they can be derived from the blood or released from macrophages that enter tissue from the blood. Other ways in which the sensitivity of nociceptors can be enhanced include a lowered pH, release of nitric oxide or other free radicals, an increased extra-cellular potassium concentration and up regulation of nerve growth factor (Kress and Reeh 1996). Nociceptor terminals are also likely to respond to neurotransmitters released in the vicinity of the nerve fibers, since a number of types of neurotransmitter receptors are found on peripheral nerve fibers (Coggeshall and Carlton 1997).

The sensitization process in primary afferent nociceptors results from the activation of second messenger systems in response to exposure of the nerve endings to inflammatory mediators (Bevan 1996). For example, bradykinin acting on the bradykinin B_2 receptor activates phospholipase C and phospholipase A_2. Phospholipase C activation triggers several intracellular metabolic cascades involving increases in the intracellular of Ca^{2+}, inositol triphosphate, diacyl-glycerol, nitric oxide and cGMP. Protein kinase C is activated and it phosphorylates a variety of proteins, including ion channel proteins. Phospholipase A_2 causes an increase in arachidonic acid and subsequently the synthesis of prostaglandins. Prostaglandins and other eicosanoids depend on synthesis

by cyclooxygenase (now separated into COX-1, the constitutive isoform and COX-2, the inducible form; McCormack 1994). Inhibition of cyclooxygenase in peripheral tissue is one mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs; Kress and Reeh 1996), although other mechanism (including a central action) are also likely (McCormack 1994). Additional second messenger system can be triggered by other agents. For example, the cAMP/protein kinase system is activated or inhibited by several different G-protein coupled receptors that interact with 5-HT, opioids and other substances.

Neurogenic inflammation depends on the release of substances from nerve endings (Geppetti and Holzer 1996). Signs of neurogenic inflammation include swelling, redness, warmth and pain (the classical signs of inflammation: tumor, rubor, calor and dolor). The first three are caused by plasma extravasation and vasodilation (Lewis 1927; Geppetti and Holzer 1996).

Central Mechanisms

Central Mechanisms of Nociceptive Transmission

Nociceptive neurons in the dorsal horn are generally classified as nociceptive-specific (NS) or as wide dynamic range (WDR) neurons, based on their responses to mechanical stimulation on skin (Mandell 1966; Willis 1985; Price 1988; Willis and Coggeshall 1991). NS neurons respond only (or primary) to noxious stimuli. WDR cells respond best to noxious stimuli, but they are also excited by the activation of sensitive mechanoreceptors, such as hair follicle afferents. Dorsal horn nociceptive neurons include interneurons and projection neuron. Nociceptive interneurons may belong to neural circuits that affect sensory processing or spinal reflexes, such as the flexor withdrawal reflex. The projection neurons transmit nociceptive information to the brain, where it is interpreted as pain. Ascending spinal cord sensory tracts that transmit somatic and visceral nociceptive information to the brain include the spinothalamic, spinoreticular, and spinomesencephalic tracts in the anterolateral quadrant and components of the postsynaptic dorsal column pathway and the spinocervical tract in the dorsal part of the cord (Willis and Coggeshall 1991). The main pathway that signals pain in humans is generally considered to be the spinothalamic tract. However, there is now evidence that, although the spinothalmic tract contributes to visceral pain, it may not be the main pathway that serves this purpose. Instead, the spinothalamic tract may play a more important role signaling cutaneous and deep somatic pain.

Prolonged or repeated noxious stimulation can result in sensitization of central nociceptive neurons; a process often termed central sensitization (Willis 1994). Central sensitization may last for

hours. A model that has proved useful for the examination of the mechanism of central sensitization is the increased responses of spinothalamic tract neurons to mechanical stimuli applied to the skin following intradermal injection of capsaicin (Simone et al. 1991; Dougherty and Willis 1992). The enhanced responses reflect enhanced responses to excitatory amino acids (Dougherty and Willis 1992) and a simultaneous reduction in responses to inhibitory amino acids (Lin *et al.* 1996b).

Central sensitization following intradermal injection of capsaicin appears to be triggered by the combined release of excitatory amino acids and substance P (SP) and perhaps other peptides (Dougherty and Willis 1991; Dougherty *et al.* 1992; 1994), resulting in the activation of several second messenger systems (Lin *et al.* 1996a; 1997; Sluka and Willis 1997). The steps leading to central sensitization include the activation of NMDA receptors, with the consequent influx of Ca^{2+} ions, and activation of G-protein coupled receptors, such as NK_1 (substance P) receptors, following by increase in levels of cyclic nucleotides, release of nitric oxide, and activation of several protein kinases, including protein kinase C, protein kinase G and protein kinase A.

Central Nervous System Mechanism of Pain Modulation

The perception of pain is evoked by stimuli that are sufficient or nearly sufficient to produce tissue damage and at least in human psychophysical studies. There is a direct relationship between stimulus intensity and reported pain intensity. This relationship can be highly variable, particularly in clinical situations. The variability depends on both peripheral and central nervous system factors. For example, pain thresholds to mechanical stimulation can be dramatically lowered in an area of inflammation by the sensitization of primary afferents. Long-lasting increases in excitability of nociresponsive dorsal horn neurons may also contribute to the lowering of pain threshold. In addition to the plasticity of afferent pain pathways that is induced by prolonged or repeated noxious stimuli, factors such as arousal, attention and emotional stress, which clearly involve central nervous system (CNS) mechanism, profoundly alter responses to noxious stimuli. For example, traumatic injuries sustained during athletic competitions or combat are often initially reported as being relatively painless. In other circumstances, these same injuries are extremely painful. The weight of evidence indicates that changes in pain responses due to arousal, attention and stress result from the action of modulatory networks that control the transmission of nociceptive messages in the CNS.

Serotonin

Serotonin (5-HT), a biogenic amine with wide distribution on both the plant and animal kingdoms, is the vasoconstrictor substance in serum which was identified, crystallized and named by Rapport *et al.* in 1948. While independently characterizing the substance that gives the enterochromaffin cells of the gastrointestinal mucosa their unique histochemical property, Erspamer (1956) found that this compound was 5-hydroxytryptamine and was identical to serotonin. The structure of 5-HT is shown in Figure [2-1]. The combination of the hydroxyl group in the 5 position of the indole nucleus and a primary amine nitrogen serving as a proton acceptor at physiological pH makes 5-HT a hydrophilic substance. As such, it does not pass the lipophilic blood brain readily. Thus, its discovery in the brain indicated that 5-HT was being synthesized in the brain, where it might play an important role in brain function. In human, 90% of the 5-HT is found in the enterochromaffin cells of the gastrointestinal mucosa. The remainder is found in platelets and the central nervous system (Sjoerdsma et al., 1970).

The serotonergic neuronal system is uniquely organize with cells of origin in the brainstem and spinal cord. In addition, there are serotonergic neurons that originate from the midbrain raphe and innervate cerebral blood vessels. When activated, these neurons change cerebral blood flow (Lance, 1992). Specific 5-HT receptor subtypes are localized to the vascular structures innervated by serotonergic neurons (Lance, 1992).

Apart from its role as neurotransmitter in the CNS, 5-HT appears to act as a modulator, altering the level of sensory responsiveness or motor activity but not actually mediating the responses (Boadle-Biber,

1993). 5-HT has been implicated in controlling feeding behavior, thermo-regulation, sexual behavior, sleep, and pain modulation (Leonard, 1992).

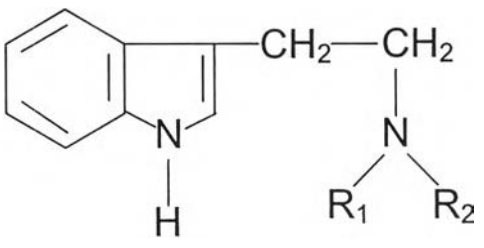


Figure [2-1] Chemical structure of 5-hydroxytrptamine (5-HT)

Serotonin Synthesis and Metabolism

Neurons and enterochromaffin cells synthesize 5-HT from the amino acid, L-tryptophan, while platelets acquire it from the blood (Sjoerdsma ed al., 1970). The biosynthesis and catabolism of 5-HT are shown in figure [2-2]. The first step in biosynthesis is catalyzed by the enzyme tryptophan 5-hydroxylase (the rate-limiting enzyme), which converts L-tryptophan to 5-hydroxytryptophan (5-HTP). 5-HTP is decarboxylated to 5-HT by the nonspecific aromatic L-amino acid decarboxylase. In neurons, 5-HT is taken up into secretory granules and stored. In man, 5-HT is mainly oxidatively deaminated by monoamine oxidase (MAO) to form 5-hydroxyindole-actetaldehyde. The aldehyde is rapidly degraded by dehydrogenase to 5-hydroxy-indoleacetic acid (5-HIAA), the major metabolite of 5-HT.

5-HT synthesis is regulated by modulating the rate of conversion of L-tryptophan to 5-HTP. The concentration of tryptophan is subsaturating for tryptophan for tryptophan hydroxylase. Administration of exogenous

tryptophan leads to a rise in brain levels of tryptophan and as increase in 5-HT synthesis in rats (Boadle-Biber, 1993). This effect depends on the rate of firing of the 5-HT neuron and does not occur if firing rates are reduced. Electrical stimulation enhances 5-HT production by increasing tryptophan hydroxylase activity, most likely by enzymes phosphorylation. Activation of somatodendritic 5-HT_{1A} auto-receptor inhibits neuronal firing and 5-HT synthesis and release of 5-HT in the absence of any effect on firing rate (Boadle-Biber, 1993).

5-HT exists in several pools, and newly synthesized 5-HT is preferentially released from the storage vesicles in response to neuronal stimulation. Many receptors have been cloned and their amino acid sequence and tertiary structure established (Peroutka, 1993).

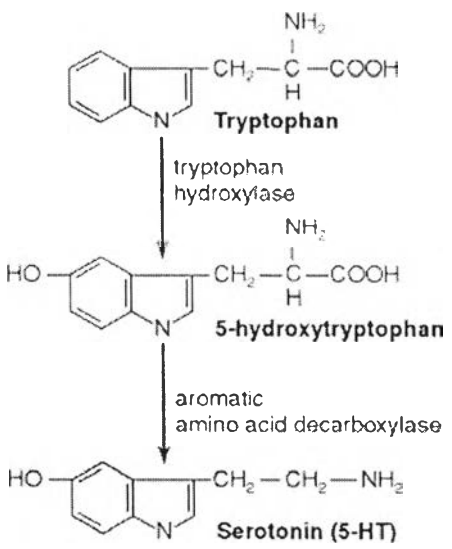


Figure [2-2] Biosynthesis of 5-hydroxytrptamine (5-HT)

The Role of 5-HT in Pain

Pain is a complex, intricate neurochemical process involving neurotransmitter and other molecules acting in both peripheral and central pain signaling pathways. Similarly, multiple neurotransmitters are involved in most neurologic and neuropsychiatric disorders including depression. Thus, several neurochemicals seem to be involved in the overlapping of the phenomena of pain and depression. 5-HT has emerged as a neurotransmitter that appears to be involved in both pain and depression. 5-HT is present in virtually every organ system in the body. Serotonergic neurons have been shown to exert an inhibitory effect on noradrenergic neurons. It has known that 5-HT pathways are present in the prefrontal cortex and the limbic system (the areas known to be involved in mood disorders, including depression).

Serotonergic neurons are also involved in a well-described pain-modulating circuit that includes the amygdala, periaqueductal gray (PAG), dorsolateral pontine tegmentum (DLPT), and rostroventral medulla (RVM). According to this model, cells in the PAG project primarily to RVM cells that in turn act on the spinal dorsal horn. When activated, RVM neurons inhibit pain sensory processing, presumably by inhibiting the dorsal horn cells that are receiving pain information. Through descending projections, this circuit controls spinal pain-signaling mechanisms as well as dorsal horn pain transmission and is an endogenous mechanism of pain relief. In multiple studies in rodents, Fields et al, 1991 have shown that 5-HT is important neurotransmitter in this pain-modulating circuit. All pain is not created equal, however. Sharp, stabbing pain and dull aching pain involve different neural pathways. Under normal circumstances, an acute pain will be transmitted to the brain and will then be resolved in part due to the endogenous pain-

modulating system. In persistent pain, however, resolution does not take place. Instead, plastic changes take place in the neural pathways involved in transmitting acute pain. Several other animal studies further support the important role of 5-HT in the process of pain modulation. Molecules that block the synthesis of 5-HT have been shown to inhibit antidepressant-mediated pain relief in mice. In related research, depletion of central serotonergic systems has been shown to block antidepressant-mediated pain relief in mice. 5-HT is likewise important for the functioning of the endogenous pain-suppressing descending projections described originally by Fields and Basbaum 1978. Normally, as part of a negative feedback loop, the output of the pain-transmission neurons helps activate the pain-suppression system. The PAG, DLPT, and RVM are the key regions of the brain involved in this descending pain modulation. Research shows that lesions made to these areas of the brain block pain relief associated with the antidepressant clomipramine. Furthermore, within the spinal cord itself, activation of serotonergic receptors also produces pain relief.

Serotonin Depletion and Painful Syndrome

Previous studies have shown that several clinical conditions are associated with low 5-HT state. For instance, migraine attacks have been shown to coincide with a decrease in platelet 5-HT level and increase in nitrate metabolites in jugular venous blood (Ferrari et al., 1989). Alteration of 5-HT has been proposed to underlie the development of fibromyalgia (FM). This hypothesis is based on the following findings: (1) reduced plasma levels of both tryptophan (TRP) and 5-HT (Stratz et al., 1993); (2) lowered levels of the 5-HT metabolite 5-HIAA in cerebrospinal fluid (CSF)(Russell *et al.*, 1992); (3) increased CSF levels of kynurenine (KYN) (Russell, 1996); (4) an inverse relationship among

levels of TRP, 5-HT, or 5-HIAA with clinical measures of pain, but a positive correlation between KYN levels and pain perception (Moldofsky, 1982; Russell et al., 1992; Schwarz et al.,1999); (5) lowered 5-HT activity by administration of para-chlorophenylalanine (PCPA; a centrally acting inhibitor of the key enzyme in 5-HT synthesis) evoking a pain syndrome similar to the clinical symptomatology of FM (Sicuteri,1992); and (6) the efficacy of tricyclic antidepressants, which act as 5-HT and norepinephrine reuptake inhibitors, in the treatment of FM (Arnold et al., 2000).

The Technique to Deplete 5-HT Level in This Study

Effects of para-chlorophyenylalanine (PCPA)

Preliminary characterization of tryptophan hydroxylase enzymes has just been completed when Koe and Weissman 1966 reported that PCPA caused a strong, long-lasting depletion of 5-HT from a variety of animal tissues. This was particularly strinking in the brain where a single dose resulted in significant depletion for periods of time up to 2 weeks. Their work showed that this compound was probably acting via inhibition of tryptophan hydroxylase. Using soluble enzyme preparations from rat brain or beef pineal, they found PCPA to be a moderately strong substrate competitive inhibitor. It did not appear, however, that this inhibition was sufficient to explain the long-lasting 5-HT depletion after a single dose of drug. An experiment was done involving *in vivo* administration of drug to rats. A single dose of 300 mg/kg body weight of PCPA was administered; the animals were sacrificed at various periods of time and the brainstem content of PCPA phenylalanine, serotonin, and tryptophan hydroxylase measured. PCPA entered the brain rapidly but within 1 day had declined

to levels that would cause no significant inhibition of the enzyme in the *in vitro* assay. The tryptophan hydroxylase levels fell rapidly but remained essentially below level of detection for approximately 4 days and then started slowly to return toward normal during the succeeding week. The changes in serotonin approximately paralleled those in tryptophan hydroxylase but were not as dramatic. In 1970, Curzon and Green were injected intraperitoneally with 100 $mgkg^{-1}$ of D-or L-PCPA and killed 24 h later. The result showed time course of the effect of both isomers on brain 5-HT and 5-HIAA levels. Brain 5-HT and 5-HIAA levels decreased maximally to about 75% of the control values 72 h after treatment and remained there for more than 144 h.

5-HT_{2A} Receptor

The 5-HT_2 receptor family currently accommodates three receptor subtypes, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, which are similar in terms of their molecular structure, pharmacology and signal transduction pathways. In recent nomenclature updates (Humphrey et al., 1993; Hoyer et al., 1994;), the 5-HT_{2A} receptor was aligned with the 5-HT D receptor (also called 5-HT_2) originally defined by Gaddum and Picarelli (1957) as mediating contractions in the guinea pig ileum. In addition, the 5-HT_{2C} appellation replaced 5-HT_{1C} to carry the latter receptor from the 5-HT_1 to the 5-HT_2 receptor family, also the 5-HT_{2B} receptor classification took on the properties of what was previously classified as the 5-HT_2-like receptor in the stomach fundus (also called 5-HT_{2F} and SRL receptor). The amino acid sequences of the 5-HT_2 receptor family have a high degree of homology within the seven trans-membrane domains but they are structurally distinct from other 5-HT receptors (Baxter et al., 1995). A characteristic of all genes in the 5-HT_2 receptor family is that they have either two introns (in the case of both the 5-HT_{2A} and 5-HT_{2B} receptors) or three introns (5-HT_{2C} receptors) in the coding sequence (Yu et al., 1991; Chen et al., 1992; Stam et al., 1992), and all are coupled positively to phospholipase C and mobilize intracellular calcium.

The receptors are well characterized at the molecular level and their distribution in the brain is established (although levels of the 5-HT_{2B} receptor seem low). The development of selective receptor antagonists is at an advanced stage but there is a need for selective agonists. Some 5-HT_2 receptor antagonists are currently undergoing clinical assessment as potential treatments for a range of CNS disorders including schizophrenia, anxiety, sleep and feeding disorders, and migraine (Baxter et al., 1995).

The brain 5-HT_{2A} receptor was initially detected in rat cortical membranes as a binding site with high affinity for [3H]-spiperone, a relatively low (micromolar) affinity for 5-HT, but with a pharmacological profile of a 5-HT receptor (Leysen et al., 1978; Peroutka and Snyder, 1979). Although this receptor was originally termed the 5-HT_2 receptor (Peroutka and Snyder, 1979), it has now been attributed to the 5- HT_{2A} receptor classification.

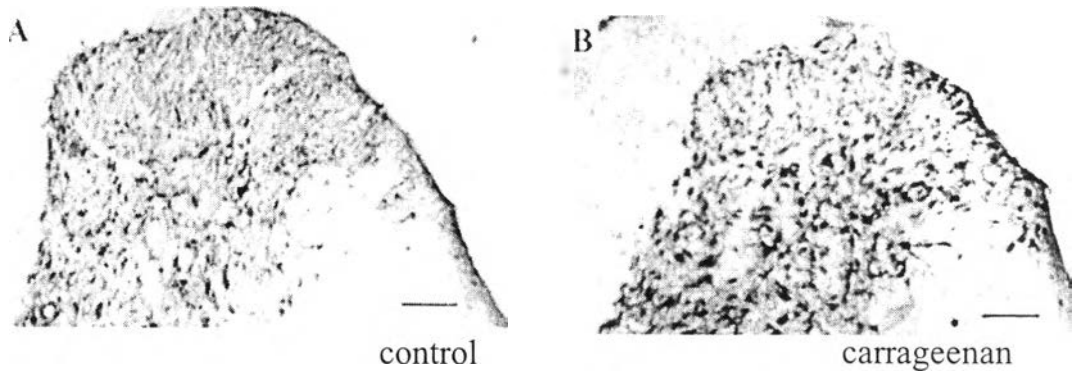
5-HT_{2A} Receptor Structure

By the mid-1980's it had already been recognized that the 5-HT_2 and 5-HT_{1C} receptors (old nomenclature) had similar pharmacological properties and second messenger systems, and that the receptors were probably structurally related. Both the rat and human 5-HT_{2A} receptor genes were isolated by homologous screening very shortly following the first reports of the 5-HT_{2C} (now 5-HT_{2C} receptor sequence (Pritchett et al., 1988; Julius et al., 1990). The human 5-HT_{2A} receptor is located on chromosome 13q14–q21 and has a relatively high amino acid sequence identity with the human 5-HT_{2C} receptor, although this is lower when compared with the human 5-HT_{2B} receptor. The human 5-HT_{2A} receptor is 87% homologous with its rat counterpart. The amino acid sequence of the 5-HT_{2A} receptor has potential sites for glycosylation (5), phosphorylation (11) and palmitoylation (1) (Saltzman et al., 1991). Experiments involving site-directed mutagenesis have identified individual amino acid residues which have major effects on the ligand binding and effector coupling properties of the 5-HT_{2A} receptor (Boess and Martin, 1994; Saudou and Hen, 1994; Baxter et al., 1995).

5-HT_{2A} Receptor Distribution

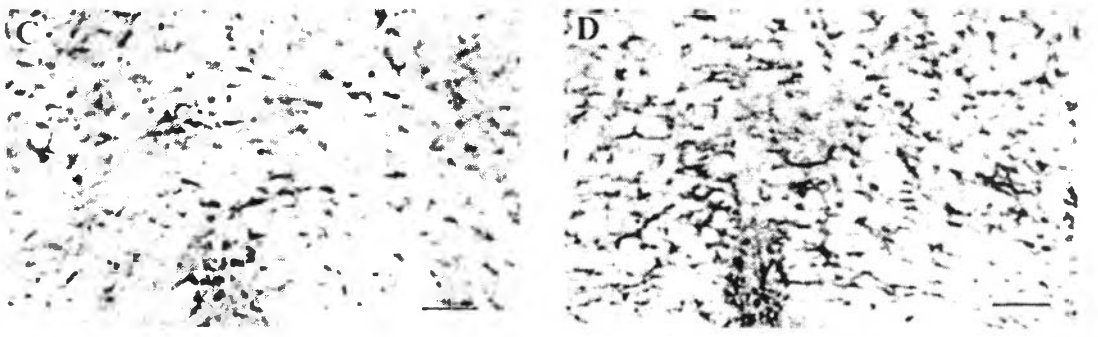
The CNS distribution of 5-HT_{2A} receptor has been mapped extensively by receptor autoradiography, *in situ* hybridisation and, more recently, immuno-cytochemistry. Receptor autoradiography studies using [3H]-spiperone, [3H]-ketanserin, [125I]-DOI and more recently [3H]-MDL 100907 as radioligands, find high levels of 5-HT_{2A} binding sites in many forebrain regions, but particularly cortical areas (neocortex, entorhinal and pyriform cortex, claustrum), caudate nucleus, nucleus accumbens, olfactory tubercle and hippocampus, of all species studied (Pazos et al., 1985, 1987; Lo´pez-Gime´nez et al., 1997). There is a generally close concordance between the distribution of 5-HT_{2A} binding sites, 5-HT_{2A} mRNA and 5-HT_{2A} receptor-like immunoreactivity (Mengod et al., 1990a; Morilak et al., 1993, 1994; Pompeiano et al., 1994; Burnet et al., 1995), suggesting that the cells expressing 5-HT_{2A} receptors are located in the region where the receptors are present (and postsynaptic to the 5-HT neuron). A number of 5-HT_{2A}-selective radioligands are currently under development for imaging 5-HT_{2A} receptors in humans, one of the most promising being the PET ligand [11C]-MDL 100907 (Lundkvist et al., 1996; Ito et al., 1998).Various studies have investigated the cellular location of the 5-HT_{2A} receptor in the brain. So far 5- HT_{2A} receptor-like immunoreactivity or 5-HT_{2A} mRNA have been found in neurons (Morilak et al., 1993, 1994; Pompeiano et al., 1994; Burnet et al.,1995), although the receptor is expressed in cultured astrocytes and glioma cells (e.g. Deecher et al., 1993; Meller et al., 1997). Converging evidence from immunocytochemical, *in situ* hybridisation and receptor autoradiography studies suggests that in various brain areas including cortex, the 5-HT_{2A} receptor is located on local (GABAergic) interneurons (Francis et al.,

1992; Morilak et al., 1993, 1994; Burnet et al., 1995; see also Sheldon and Aghajanian, 1991). Recent data from *in situ* hybridisation studies also indicate the presence of 5-HT_{2A} receptor in cortical pyramidal (projection) neurones (Burnet et al., 1995; Wright et al., 1995), which are known to be glutamatergic. It is reported that 5-HT_{2A} receptor-like immunoreactivity may be located in cholinergic neurons in the basal forebrain and specific nuclei in the brain stem (Morilak et al., 1993). The study of Zhang et al. in the year 2001 showed the up-regulation of 5-HT_{2A} receptor in carrageenan-induced inflammation in pain-related areas in CNS by using *in situ* hybridization technique.



Ipsilateral lumbar dorsal spinal horn

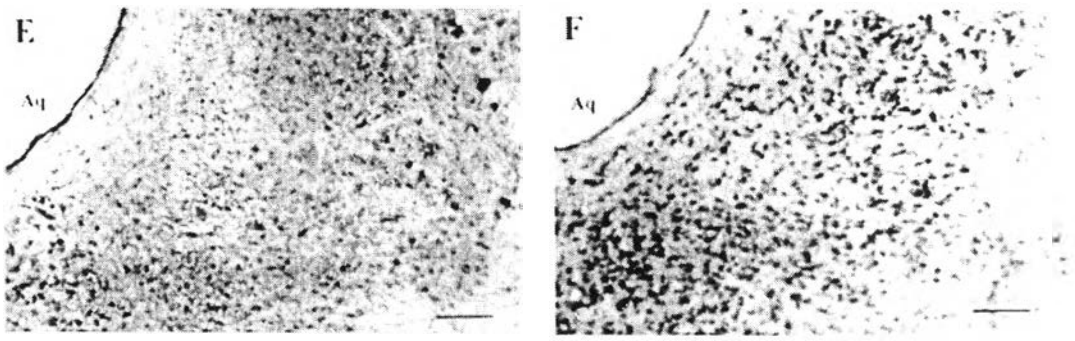
Figure [2-3] The expression of 5-HT_{2A} receptor mRNA in rats' spinal cord (L4-L5) 3 hours after carrageenan injection to the rats' hind paw



control carrageenan

Ipsilateral Nucleus Raphe Magnus

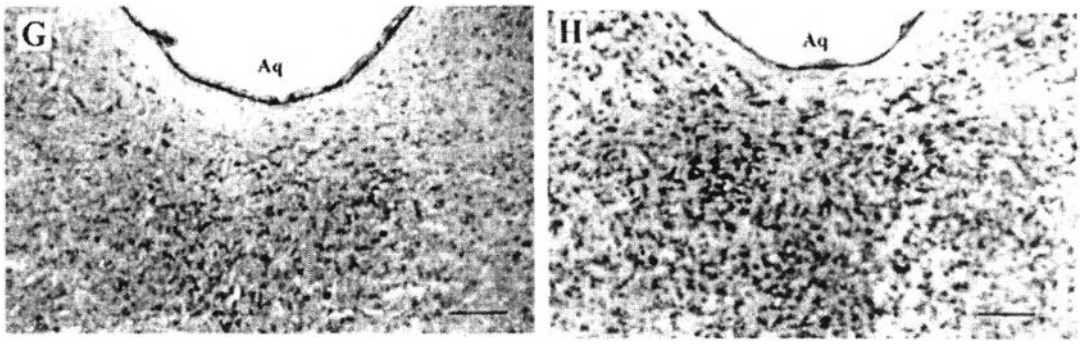
Figure [2-4] The expression of 5-HT_{2A} receptor mRNA in rats' ipsilateral nucleus raphe magnus 3 hours after carrageenan injection to the rats' hind paw.



control carrageenan

Ipsilateral ventrolateral Periaqueductal Gray

Figure [2-5] The expression of 5-HT_{2A} receptor mRNA in rats' ventrolateral periaqueductal gray 3 hours after carrageenan injection to the rats' hind paw.



control carrageenan

Bilateral Dorsal Raphe Nucleus

Figure [2-6] The expression of 5-HT_{2A} receptor mRNA in rats' bilateral dorsal raphe nucleus 3 hours after carrageenan injection to the rats' hind paw.

It has been noted that the distribution of 5-HT_{2A} binding sites appears to map onto the distribution of 5-HT axons arriving from the DRN (Blue et al., 1988). For example in the rat, the DRN 5-HT innervation of the frontal cortex seems to follow the laminar distribution of 5-HT_{2A} binding sites in this region. That 5-HT_{2A} receptors receive a selective innervation from DRN, however, may not generalize to other regions. Thus, it has been found in electrophysiological experiments that 5-HT_{2A} receptor-mediated responses in the prefrontal cortex can be evoked by stimulation of the MRN

5-HT_{2A} Receptor Pharmacology

The 5-HT_2 family of receptors is characterized by a relatively low affinity for 5-HT, a high affinity for the 5-HT2 receptor agonist, DOI, and its structural analogues (DOB, DOM), and a high affinity for various 5-HT_2 receptor antagonists, including ritanserin and ICI 170809. Until recently, it has been difficult to discriminate between the 5-HT_2 family members; although ketanserin and spiperone are about two orders of magnitude more selective for the 5-HT_{2A} versus 5-$HT_{2B:2C}$ receptors, these drugs have affinity for other monoamine receptors. However, a number of selective antagonists are now available which greatly aid the delineation of the 5-HT_2 receptors in both *in vitro* and *in vivo* models (Baxter et al., 1995). MDL 100907 is a newly developed, potent and selective antagonist of the 5-HT_{2A} receptor which has lower affinity for the 5-HT_{2C} receptor or other receptors (Sorensen et al., 1993; Kehne et al., 1996). Discrimination of the 5-HT_{2A} receptor from other members of the 5-HT_2 receptor family has also become considerably more straightforward by the development of antagonists which distinguish between 5-HT_{2A} and 5-$HT_{2C:2B}$ receptors (SB 200 646A and SB 206 553) and more recently by the arrival of potent antagonists with selectivity for both 5-HT_{2B} (SB 204 741) and 5-HT_{2C} receptors (SB 242 084 and RS-102 221) (Baxter et al.,1995; Baxter, 1996; Kennett et al., 1996a,b, 1997a,b; Bonhaus et al., 1997). At present, there is no suitably selective agonist for the 5-HT_2 receptor subtypes although certain tryptamine analogues (in particular BW 723C86 and 5-methoxytryptamine) have some selectivity for the 5- HT_{2B} receptor in *in vitro* preparations (Baxter et al., 1995; Baxter, 1996). An agonist, RO 60-0175, with selectivity for the 5-HT_{2C} receptor was recently reported (Millan et al., 1997).

Functional Effects Mediated via The 5-HT_{2A} Receptor

Second Messenger Responses

All three 5-HT_2 receptor subtypes couple positively to phospholipase C and lead to increased accumulation of inositol phosphates and intracellular Ca^{2+} (Boess and Martin, 1994; Sanders-Bush and Canton, 1995). Stimulation of the 5-HT_{2A} receptor has been demonstrated to activate phospholipase C in both heterologous expression systems (Pritchett et al.,1988a,b; Julius et al., 1990; Stam et al., 1992) and brain tissue (Conn and Sanders-Bush, 1984; Godfrey et al., 1988), via G-protein coupling (Sanders-Bush and Canton,1995). In these second messenger studies, DOI, DOB and DOM (and LSD) have partial agonist properties (Sanders-Bush et al., 1988). The non-selective 5-HT_2 receptor agonists, mCPP and TFMPP, have even lower efficacy and usually display only 5-HT_{2A} receptor antagonist activity in functional models (Conn and Sanders-Bush, 1986; Grotewiel et al., 1994). All 5-HT_2 receptors desensitize following prolonged exposure to 5-HT and other agonists (Sanders-Bush, 1990), although the sensitivity to agonists and mechanisms underlying desensitization of each subtype (particularly 5-HT_{2A} versus 5-HT_{2C}) may be different (Briddon et al., 1995). A curious property of 5-HT_{2A} receptors is that in some *in vitro* and *in vivo* models they down-regulate in the face of constant exposure to certain antagonists (mianserin, spiperone and mesulergine) (e.g. Sanders-Bush, 1990; Roth and Ciaranello, 1991; Grotewiel and Sanders-Bush, 1994). One of several explanations put forward to account for this phenomenon is that under certain conditions, 5-HT_{2A} receptors are constitutively active, and that some of the ligands act as inverse agonists. Of current interest is evidence that stimulation of the 5-HT_{2A} receptor causes activation of a biochemical

cascade leading to altered expression of a number of genes including that of brain-derived neurotrophic factor (BDNF) (Vaidya et al., 1997). These changes may be linked at least in part to the increase in expression of BDNF seen following repeated treatment with antidepressants (Duman et al., 1997). There is the exciting but as yet unproven possibility that the latter changes lead to altered synaptic connectivity in the brain, and that this may even contribute to the therapeutic effect of antidepressants.

Electrophysiological Responses

5-HT_2 receptor activation results in neuronal excitation in a variety of brain regions. Although few of these responses have been analyzed using newly available 5-HT_2 receptor subtype-selective agents, evidence suggests that in some of these cases the responses are mediated by the 5-HT_{2A} receptor while others involve the 5-HT_{2C} receptor (Aghajanian, 1995). Clear evidence for a 5-HT_{2A} receptor-mediated excitation in the cortex comes from intracellular recordings of interneurons in slices of rat pyriform cortex. Thus, the 5-HT-induced activation of these cells is blocked by both selective (MDL 100907) and non-selective 5-HT_{2A} receptor antagonists (Sheldon and Aghajanian, 1991; Marek and Aghajanian, 1994). Furthermore, LSD and DOI are potent but partial agonists in this preparation (Marek and Aghajanian, 1996). 5-HT-induced neuronal depolarizations have also been detected in slice preparations of the nucleus accumbens (North and Uchimura, 1989), neocortex (Araneda and Andrade, 1991; Aghajanian and Marek, 1997), dentate gyrus of the hippocampus (Piguet and Galvan, 1994), and all have the pharmacological characteristics which bear the hallmark of the 5-HT_{2A} receptor. The excitatory responses to 5-HT_{2A} receptor activation are associated with a reduction of potassium conductances (Aghajanian

1995), although whether the phosphoinositide signaling pathway has a role in this effect is not certain. Electrophysiological studies also implicate the 5-HT_2 receptor in the regulation of noradrenergic neurons in the locus coeruleus (LC). Evidence from recordings in anaesthetized rats suggests that 5-HT_2 receptor activation results in both the facilitation of sensory-evoked activation of noradrenergic neurons, and inhibition of their spontaneous activity (Aghajanian, 1995). The inhibitory effect of 5-HT_2 receptor activation on noradrenergic transmission has also been detected in microdialysis studies which demonstrate a decrease in noradrenaline release in rat hippocampus following administration of DOI and DOB, and the reversal of this effect by ritanserin and spiperone (Done and Sharp, 1992). There is evidence from microdialysis studies in the awaked rat that 5-HT_2 receptor antagonists increase noradrenaline release (Done and Sharp, 1994). Although earlier data indicates that the pharmacology of the 5-HT_2 receptor modulating noradrenaline is of 5-HT_{2A} subtype, this idea needs reappraisal in view of new findings that $5HT_{2C}$ antagonists increase nonadrenaline in microdialysis experiments (Millan et al., 1998) The effects of 5-HT_2 receptor activation on noradrenergic neurons are likely to be indirect, possibly involving afferents to the LC from the brain stem (Gorea et al., 1991; Aghajanian, 1995). Interestingly, there is evidence for a 5-HT2 receptor-mediated excitation of neurons in the nucleus prepositus hypoglossi which is a major source of inhibitory input to the LC (Bobker, 1994).

Behavioral and Other Physiological Responses

The behavioral effects of 5-HT_2 receptor agonists in rodents are many, ranging from changes in both unconditioned (e.g. increased motor activity and hyperthermia) and conditioned responses (e.g. punished responding, drug discrimination) (Glennon and Lucki, 1988; Koek et al., 1992). The delineation of the involvement of specific 5-HT_2 receptor subtypes in these behaviors has not been straightforward due to the fact that most 5-HT_2 receptor agonists studied so far, are not selective. Nevertheless certain behaviors can be attributed, with some degree of confidence, to activation of either 5-HT_{2A} or 5-HT_{2C} receptors. Head twitches (mice) and wet dog shakes (rats) induced by drugs such as DOI and its structural analogues, as well as 5-HT releasing agents and precursors like 5-HTP, have long been thought to be mediated via a receptor of the 5-HT_2 type Green and Heal, 1985). It now seems clear that this response is 5-HT_{2A} receptor-mediated. Thus, the potency with which 5-HT_2 antagonists inhibit agonist-induced head shakes closely correlates with their affinity for the 5-HT_{2A} binding site but not other binding sites, including the 5-HT_{2C} binding site (Arnt et al., 1984; Schreiber et al., 1995). Furthermore, 5-HT_{2A} receptor selective antagonists such as MDL 100907 inhibit the head shake response while 5-$HT_{2B:2C}$ receptor selective antagonists (SB 200 646A) do not (Kennett et al., 1994; Schreiber et al., 1995). It should be pointed out, however, that the use of this model as an *in vivo* test of 5-HT_{2A} receptor pharmacology is complicated by the fact it is sensitive to drugs active on other transmitter receptors (5-HT_1 and catecholamine receptors, in particular) which presumably interact indirectly with the neural pathways expressing the headshake: twitch response (Koek et al., 1992).

Activation of the 5-HT_2 receptor leads to a discriminative stimulus

in rats. For example, animals trained to discriminate 5-HT_2 receptor agonists such as DOM, recognize its structural derivatives (DOI, DOB) but no 5-HT_1 receptor agonists (Glennon and Lucki, 1988). The DOM stimulus is blocked by 5-HT_2 receptor antagonists such as ketanserin and LY 53857, suggesting that the discriminative cue is 5-HT_{2A} receptor-mediated. Recent data show that the potency of 5-HT_2 receptor antagonists to block the DOM cue correlates strongly with their affinity for the 5-HT_{2A} but not 5-HT_{2C} binding site (Fiorella et al., 1995). In addition there is a significant correlation between the potency of a wide range of 5-HT_2 receptor agonists in the DOM discrimination model and their affinity for the 5-HT_{2A} binding site (Glennon, 1990). The non-selective 5-HT_2 receptor agonist, mCPP, also evokes a discriminative stimulus in rats, but this appears to involve a dopaminergic mechanism and not 5-HT_2 or other 5-HT receptors (Bourson et al., 1996).

An agonist action at 5-HT_2 receptors is likely to be involved in hallucinogenic mechanisms since there is a close correlation between the human hallucinogenic potency of 5-HT_2 receptor agonists and their affinity for the 5-HT_2 binding sites (Glennon, 1990). Although this correlation fitted best for the 5-HT_{2A} binding site, 5-HT_{2C} sites were also strongly correlated. Despite the latter correlation, it has been argued that the 5-HT_{2C} receptor may not be important as mCPP, which acts as a 5-HT_{2C} agonist in many models is not an hallucinogen in humans. However, this argument is complicated by the fact that mCPP has 5-HT_{2A} receptor antagonist properties in some models. Currently there is considerable interest in the role of the 5-HT_{2A} receptor in antipsychotic drug action. This interest is based on many findings including the relationship between 5-HT_{2A} receptor and hallucinogens discussed above; also clozapine, olanzepine and other atypical antipsychotic drugs have high affinity for the 5-HT_{2A} binding site e.g. (Leysen et al., 1993), and the

evidence of an association between schizophrenia and treatment outcome and certain polymorphic variants of the 5-HT_{2A} receptor (Busatto and Kerwin, 1997). Moreover, selective 5-HT_{2A} receptor antagonists (especially MDL 100907) appear to be active in animal models predictive of atypical antipsychotic action (Kehne et al., 1996). Whether selective 5-HT_{2A} receptor antagonists are as clinically effective as antipsychotics compared to the available mixed 5-HT_2:dopamine receptor antagonists (e.g. sertindole, risperidone) is clearly a critical question. Finally, other responses to 5-HT_2 receptor agonists that may be mediated by the 5-HT_{2A} receptor include hyperthermia (Gudelsky et al., 1986), and neuroendocrine responses such as increased secretion of cortisol, ACTH, renin and prolactin (e.g. Fuller, 1996; Van de Kar et al., 1996). The functional effects associated with activation of central 5-HT_{2A} receptors are summarized in Table [2-2].

Table [2-1] Affinity (p*Ki*) of various ligands for 5-HT_2 receptors

	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
5-HT_{2A} receptor			
Spiperone	8.8	5.5	5.9
MDL 100 907	9.4	n.d.	6.9
Ketanserin	8.9	5.4	7.0
5-HT_{2B} receptor			
5-MeOT	7.4a	8.8a	6.2a
a-Methyl-5-HT	6.1a	8.4a	7.3a
SB 2044741	B5.3	7.8	B6.0
BW 723C86	B5.4a	7.9a	B6.9
5-HT_{2C} receptor			
SB 242084	6.8	7.0	9.0
RS–102221	6.0	6.1	8.4
RO 60-0175	6.0	5.8	8.8
5-$HT_{2B:2C}$ receptors			
SB 200646A	5.2	7.5	6.9
mCPP	6.7	7.4a	7.8
SB 206553	5.8	8.9	7.9
Non-selective			
LY 53857	7.3	8.2	8.1
ICI 170809	9.1	n.d.	8.3
Ritanserin	8.8	8.3	8.9
Mianserin	8.1	7.3	8.0
DOI	7.3a	7.4a	7.8a

a pEC50 value for agonist. 5-MeOT, 5-methoxytryptamine; n.d., not determined. Data were taken from Baxter et al. (1995) with additions from Bonhaus et al. (1997), Millan et al. (1997) and Kennett et al. (1996a,b, 1997a,b).

Table [2-2] Summary of the functional responses associated with activation of the brain 5-HT_{2A} receptor

Level	Response	Mechanism
Cellular	Phosphatidyl inositide turnover	Post
Electrophysiological	Neuronal depolarisation	Post
Behavioral	Head twitch (mouse)	Post
	Wet dog shake (rat)	Post
	Hyperthermia	Post
	Discriminative stimulus	Post
Neurochemical	Noradrenaline release	Post
Neuroendocrine	Cortisol	Post
	ACTH	Post

Behavioral Studies of 5-HT_{2A} Receptor in Pain

Several studies showed the controversial roles of 5-HT_{2A} receptor in painful state. From the studies, there may classify the painful condition into 2 models as inflammatory and neuropathic pain models.

Inflammatory Pain Models

In year 2001, Kjørsvik et al. reported that activation of spinal serotonin$_{2A/2C}$ receptor (5-$HT_{2A/2C}$ receptor) augments nociceptive response in the rats. They used formalin test to determine the effect of 5-$HT_{2A/2C}$ agonist, (+)-1-2-(2, 5-dimethoxy-4-iodophenyl)-2 aminopropane (DOI, 0.01 mM in 15 µl) or antagonist, ketanserin 0.1 mM in 15 µl. They found an increased of both early and late phase of formalin involve pain-like behavior in DOI treatment, while ketanserin treatment suppressed those behaviors. Therefore, they suggested that 5-$HT_{2A/2C}$ receptor increases the spinal afferent nociceptive impulse induced by peripheral inflammation.

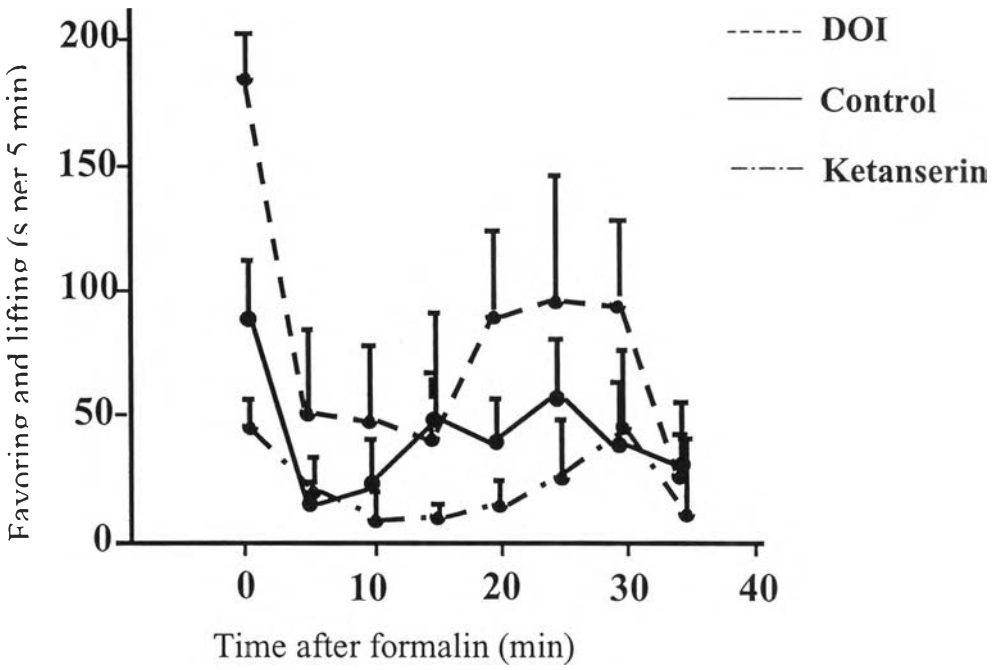


Figure [2-7] The effect of 5-HT_{2A} agonist (DOI) or antagonist (Ketanserin) on favoring and lifting behaviors in formalin test.

In addition, Okamoto et al., (2002) investigated the effect of peripheral inflammation on the thermal hyperalgesia and the role of 5-HT_{2A} receptor in inflammatory pain condition. The complete Freund's adjuvant (CFA) was introduced into hind paw. The paw withdrawal test was conducted for 18 days. It was found that the oral administration of 5-HT_{2A} receptor antagonist; Sarpogrelate HCl could reduce the difference of the paw withdrawal latency between the hind limbs.

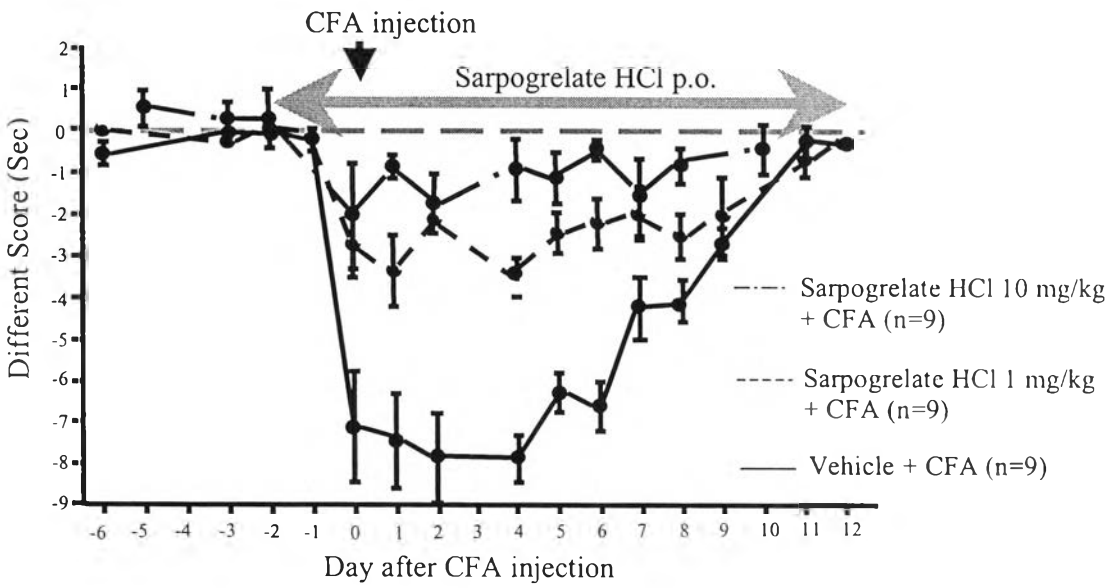


Figure [2-8] The effect of 5-HT_{2A} receptor antagonist, Sarpogrelate HCl, on thermal hyperalgesia using the paw withdrawal test after CFA-induced peripheral inflammation.

Neuropathic Pain Models

It was found the controversial role of 5-HT_{2A} receptor in neuropathic pain condition. Obata et al. (2001) used the chronic spinal nerve ligation as a model of neuropathic pain. The Von Frey hair test was performed to determine the response of the mechanical threshold. This test reflected the allodynic condition of the animals. The spinal nerves (L5-L6) were ligated for 10 days. They found that intrathecally administration of DOI (10-100 μg) increased the mechanical threshold in dose dependent manner.

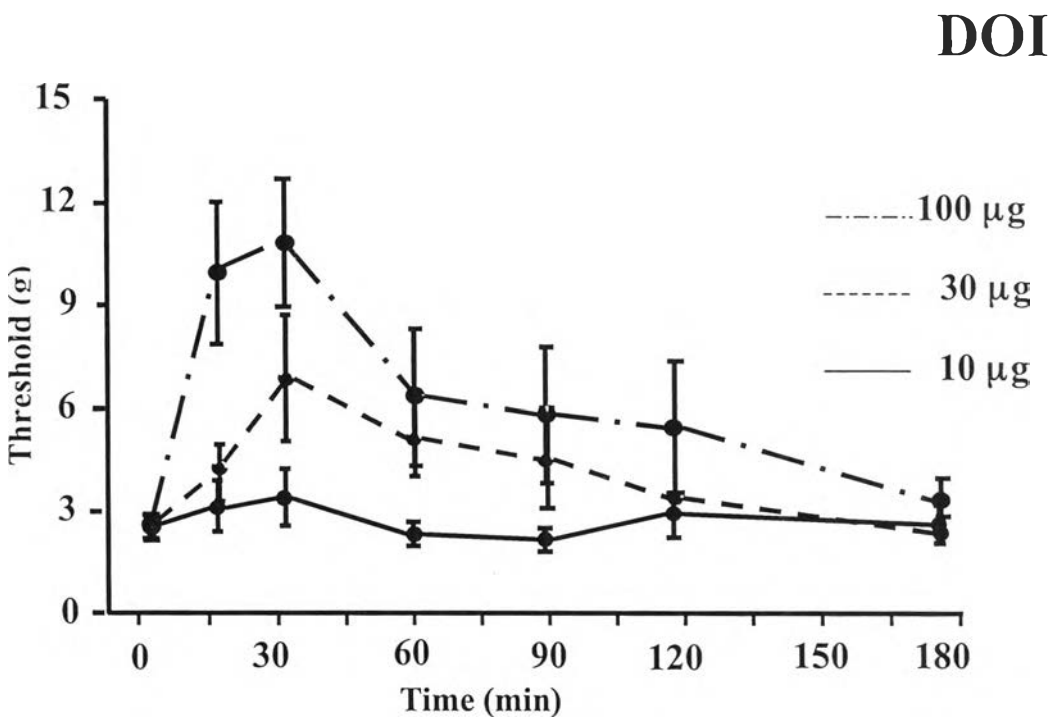


Figure [2-9] The effect of DOI, 5-HT_{2A} agonist on mechanical threshold in Von Frey hair test after the chronic nerve ligation.

Moreover, Okamoto et al., (2002) also performed the chronic constriction injury (CCI)-induced neuropathic condition to investigate the effect of peripheral inflammation on the thermal hyperalgesia and the role of 5-HT_{2A} receptor. They found that Sarpogrelate HCl failed to alter the paw withdrawal latency between the hind limbs. They suggested that 5-HT_{2A} receptor does not have a role in thermal hyperalgesia in neuropathic pain condition.

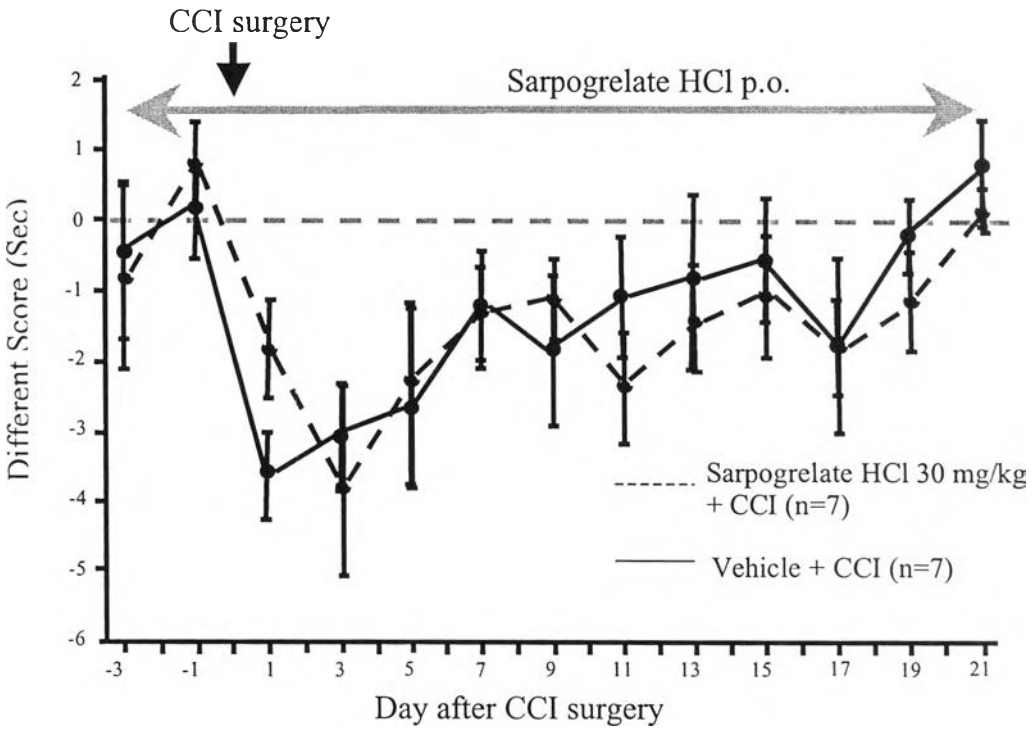


Figure [2-10] The effect of Sarpogrelate HCl, 5-HT_{2A} antagonist on the thermal hyperalgesia using paw withdrawal test after the chronic constriction injury model.

Behavioral Models of Nociception

The study of pain in awake animals raises ethical, philosophical, and technical problems. Philosophically, there is the problem that pain cannot be monitored directly in animals, but can only estimate by examining their responses to nociceptive stimuli; however, such responses do not necessarily mean that there is a concomitant sensation. The types of nociceptive stimuli (electrical, thermal, mechanical, or chemical) that have been used in different pain models are accepted that none is ideal (Le Bars *et al.*, 2001) However chemical stimuli probably most closely mimic acute clinical pain.

Behavioral studies of pain are distinguished into the stimulus and the response. Chemical stimulation involving the administration of algogenic agent represents a slow or very slow, form of stimulation. In this respect, chemical stimuli are clearly different from other forms of stimulation. They are progressive, are of longer duration, and have an inescapable character once they have been applied. As a result, typical reflexes which necessitate a minimum level of synchronization of activity in primary afferent, are not produced by these stimuli (Gilchrist *et al.*, 1996; Yeomans *et al.*, 1996)

The responses of the stimuli are wide spectrum ranging due to the optimum. The ranging are from the most elementary reflexes to far more integrated behaviors such wound licking, escape or avoidance. In almost every case, it is a motor response that is monitored.

The measurements of pain level may be classified into two major groups; use of short-duration stimuli (Phasic pain) and of long-duration stimuli (Tonic pain). The Phasic pain is commonly used, and can be classified by the nature of the stimulus, be it thermal, mechanical, or electrical.

The Test Based on the Short-duration Stimuli

In this chapter, it only mentioned the test based on the thermal stimuli. The test based on the use of thermal stimuli are widely used. In tests involving thermal stimuli, it is always the skin that is stimulated. These tests do not involve visceral or musculoskeletal tissues. However, it is important that radiant heat also stimulates thermo-receptor, and that, consequently, the application of a ramped thermal stimulus will result in an organized and unalterable sequence of activation, namely thermo-receptors, then thermo-receptor plus nociceptors, then nociceptors alone, and finally (possibly) nociceptors plus "paradoxical cold" receptors (Le Bars, 2001) Figure [2-3]

In practice, the animal withdraws itself quickly from the stimulus, and therefore only the first part of this scenario takes place. The source of nociceptive stimulation can be distant from its target (e.g., radiant heat from a lamp) or can be in direct contact with the skin. Radiant heat constitutes a relatively selective stimulus for nociceptors and has an advantage over the other modes of thermal stimulation in that it produces no tactile stimulus.

Three tests based on thermal stimuli (hot plate, tail flick and paw withdrawal test) are well-known. The suitable test for peripheral inflammatory pain model is paw withdrawal test which was adapted from the tail-flick test.

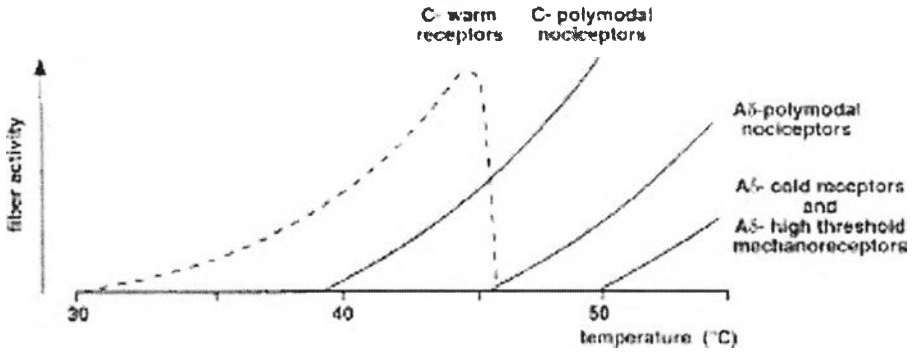


Figure [2-11] Diagrammatic representation of the activity evoked in cutaneous receptors by different temperatures applied to the skin (Hensel, 1973; Duclaux and Kenshalo, 1980; Meyer *et al.*, 1994; Treede *et al.*, 1995). When the temperature is gradually increased from the normal value for the skin (around 30°C with an ambient temperature of 20°C) to within the noxious range, there is a successive activation of thermo-receptors and then C- and A -polymodal nociceptors. At the highest temperatures, high-threshold mechano-receptors and cold receptors are also activated. On the basis of the recruitment of these different receptors, four successive periods shown in A, B, and C can be defined: 1) thermo-receptors; 2) thermo-receptors and C-polymodal nociceptors; 3) C- and A -polymodal nociceptors; and 4) polymodal nociceptors, high-threshold mechanoreceptors, and cold receptors.

In 1941 D'Amour and Smith applied thermal radiation to the tail of an animal and it provoked the withdrawal of the tail by a brief vigorous movement. This method was modified by Hargreaves and co-workers in 1988. Hide paws of the animals were used instead of the tail. This test offers the advantage that it does not involve the pre-eminent organ of thermoregulation in rats and mice, i.e. the tail. One can improve the test by minimizing variations in the baseline temperature of the skin (Galbraith et al., 1993; Dirig et al., 1997). With the aim of studying

hyperalgesic phenomena resulting from inflammation, Hargreaves et al. (1988) had an inspired idea for supplementing the model of Randall and Selitto (1957): radiant heat was applied to a paw that had already been inflamed by a subcutaneous injection of carrageenin. For this purpose, inflammation can also be produced by exposure to ultraviolet rays (Perkins et al., 1993). One advantage in these tests is that heat is applied (to the plantar surface of the foot) of a freely moving animal. However, there is a disadvantage in that the position of the leg becomes a factor since the background level of activity in the flexors varies with the position of the animal.

Yeomans and Proudfit (1994, 1996) and Yeomans et al. (1996b) studied the withdrawal of the hind paw in the anesthetized rat and came to the following conclusions: when the heating slope is steep (6.5°C/s), the paw withdrawal reaction time is short and the skin surface temperature reaches a high level, suggesting A fibers are activated; when the heating is slow (1°C/s), the reaction time is longer and skin temperature increases less, activating only C fibers. Morphine is far more active in the second than in the first of these tests (Lu et al., 1997).

The Test Based on the Long-duration Stimuli

Basically, these tests involve using an irritant, algogenic chemical agent as the nociceptive stimulus. They differ from the vast majority of other tests in that they abandon the principle of determining the nociceptive threshold and involve a quantitative approach to the behavior observed after the application of a stimulus with a potency that is going to vary with time. They can be thought of as a kind of model for tonic pain. However, they are not models for chronic pain because their duration is only in the order of some tens of minutes.

The main types of behavioral test based on such stimuli use intradermal or intraperitoneal injections. The use of intra-arterial or intradental bradykinin is less common (Guzman et al., 1964; Deffenu et al., 1966; Lim and Guzman, 1968; Foong et al., 1982), although intracapsular (jaw) injections of algogenic substances have also been used recently in pharmacological studies of pain in nonbehavioral models in which the animals are anesthetized (Broton and Sessle, 1988; Yu et al., 1994, 1995, 1996). In addition, there are behavioral tests that use the intracapsular administration of urate crystals, Freund's adjuvant, or carrageenin, but these are related to models of chronic inflammatory pain (Okuda et al., 1984; Otsuki et al., 1986; Coderre and Wall, 1987; Butler et al., 1992; Tonussi and Ferreira, 1992).

The most commonly used substance for intradermal injections is formalin (the "formalin test"). The term *formalin* usually means a 37% solution of formaldehyde.

Less commonly used are hypertonic saline (Lewis and Kellgren, 1939; Hwang and Wilcox, 1986), ethylene diamine tetra-acetic acid (Teiger, 1976), Freund's adjuvant (Iadarola et al., 1988), capsaicin (Sakurada et al., 1992), and bee sting (Larivie`re and Melzack, 1996).

Other substances have been tested but with less success (Wheeler-Aceto et al., 1990).

A 0.5 to 15% solution of formalin injected into the dorsal surface of the rat forepaw provokes a painful behavior that can be assessed on a four-level scale related to posture: 0, normal posture; 1, with the injected paw remaining on the ground but not supporting the animal; 2, with the injected paw clearly raised; and 3, with the injected paw being licked, nibbled, or shaken (Dubuisson and Dennis, 1977). The response is given a mark, and the results are expressed either continuously per unit of time or at regular time intervals when several animals are observed sequentially (Abbott et al., 1999). Each level on this scale can be weighted to optimize the test (Coderre et al., 1993; Abbott et al., 1995; Watson et al., 1997). This method has also been used in the mouse, cat, and monkey (Dubuisson and Dennis, 1977; Alreja et al., 1984; Hunskaar et al., 1985; Murray et al., 1988; Tjølsen et al., 1992). The measured parameter can also be the number of licks or twitches of the paw per unit of time (Wheeler-Aceto and Cowan, 1991), the cumulative time spent biting/licking the paw (Sufka et al., 1998), or even a measure of the overall agitation of the animal obtained by a strain gauge coupled to the cage (Jett and Michelson, 1996). Such specific behaviors resulting from an injection of formalin can be captured automatically by a camera attached to a computer; in this way, the effects of a pharmacological substance on such motor activity can be identified, analyzed, and uncoupled from antinociceptive effects (Jourdan et al., 1997). This test has been adapted for use in the trigeminal region (Clavelou et al., 1989, 1995).

In the rat and the mouse, intraplantar injections of formalin produce a biphasic behavioral reaction. This behavior consists of an initial phase, occurring about 3 min after the injection, and then after a

quiescent period, a second phase between the 20th and 30th minutes. The intensities of these behaviors are dependent on the concentration of formalin that is administered (Rosland et al., 1990; Aloisi et al., 1995; Clavelou et al., 1995). The first phase results essentially from the direct stimulation of nociceptors, whereas the second involves a period of sensitization during which inflammatory phenomena occur. The central or peripheral origin of this second phase has been the subject of debate (Tjølsen et al., 1992). For some, the second phase results from central processes triggered by the neuronal activation during the first phase (Coderre et al., 1993). However, this hypothesis seems unlikely not only because formalin provokes biphasic activity in afferent fibers (McCall et al., 1996; Puig and Sorkin, 1996), but even more so because the blocking of the first phase by substances with rapid actions (e.g., subcutaneous lidocaine or intravenous remifentanil) does not suppress the second phase (Dallel et al., 1995; Taylor et al., 1995, 1997). Thus, the second phase cannot be interpreted as a consequence of the first; it clearly also originates from peripheral mechanisms.

Opioid analgesics seem to be antinociceptive for both phases, although the second is more sensitive to these substances. In contrast, NSAIDs such as indomethacin seem to suppress only the second phase (Hunskaar and Hole, 1987; Shibata et al., 1989; Malmberg and Yaksh, 1992; Jourdan et al., 1997), especially when the formalin is injected in high concentrations (Yashpal and Coderre, 1998).

Another model of tonic cutaneous pain has been proposed recently. This test involves mimicking postoperative pain triggered by a cutaneous incision (Brennan et al., 1996; Zahn et al., 1997).

c-fos as an Indicator of Neuronal Activity

c-fos gene and its product, Fos protein are involved in the signal transduction cascade that is responsible for the intracellular changes provoked by extracellular events. One possible way of mechanism is that Fos may contribute to long-term modulation of spinal nociceptive processes by involvement in the changes in spinal nociceptive circuits that lead to increased sensitivity to noxious stimuli (''hyperalgesia'') or non-noxious stimuli (''allodynia'') (Harris A, 1998).

Neurons expressing *c-fos* typically locate in laminae I and II, and laminae V and VI of the dorsal horn, which neurons correspond to the terminal fields of primary nociceptive afferent fibers and to the distribution of noci-responsive neurons. In 1996, Ma and Woolf investigated changing in spinal *c-fos* expression in rats given an injection of CFA. Rats treated with CFA showed pain-related response to non-noxious stimuli applied to the injected paw. Interestingly, in treated rats, the level and laminar distribution of *c-fos* expression induced by non-noxious stimulation was reported to resemble that induced by noxious stimuli. In contrast, it was found that analgesic drugs including morphine suppressed the expression of *c-fos* in spinal cord (Presley et al., 1990). From the evidence above, it might claim that *c-fos* has a role in spinal nociceptive transmission.

To investigate nociceptive processing, studies of *c-fos* expression focused on not only spinal cord, but also in the brain. Bullitt (1989) examined Fos expression in the brainstem, hypothalamus, and thalamus in rats subjected to noxious mechanical stimulation or noxious thermal stimulation. It was found that these forms of noxious stimulation induced Fos expression in the dorsal reticular nucleus, central gray, dorsal raphe,

parabrachial nucleus, inferior colliculus, lateral hypothamus, and the midline and intralaminar thalamic nuclei.

Each of these studies has confirmed the relationship between nociception and *c-fos* expression, thus adding to the body of evidence establishing (Harris, 1998) this as a valid tool for the study of the neural correlates of nociception.

In summary, the following experiments were used three procedures to determine the roles of 5-HT_{2A} receptor in pain pathways.

1. Observed behavioral study

This procedure aims at determining the complex behaviors of the rats. The expression of those behaviors may implicate the overall physiological mechanism.

2. Paw withdrawal test

This procedure aims at determining the spinal reflex. The paw withdrawal latency determines the tolerance to noxious heat stimuli.

3. Immunohistochemical Study

This procedure aims at determining the cortical activity. The number of Fos expression may indicate the central sensitization.