Chapter I

Introduction

Mitragynine

Mitragynine is an indole alkaloid found as major alkaloid of *Mitragyna speciosa* (Korth.). This alkaloid has been isolated among 24 others by groups of investigators (Jansen and Prast, 1988). *Mitragyna speciosa* (known in Thai as "Kratom" and in Malaya as "Biak") is a South-East Asian tree, the leaves of which provide several alkaloids of considerable interest and seemingly contradictory properties. The tree is 10-25 meters in height. The leaves are elliptic, 8.5-14 cm long, 5-10 cm wide (smaller at the ends of branchlets) pointed at the tip, rounded or somewhat heart-shaped at the base, and hairy on the nerves beneath. The petioles are 2-4 cm long. The flowers are crowded in round, terminal inflorescences (heads) 3-5 cm long. The calyx tube is short and cup shaped, with rounded lobes. The corolla tube is 5 mm long, smooth, and revolute in the margins. The fruit is oblong-ovoid and 5-7 mm long, with 10 ridges (Quisumbing, 1951). Drawing of *Mitragyna speciosa* is shown figure 1.

The leaves of *Mitragyna speciosa* (Korth.) have long been known to possess narcotic properties. They chewed, smoked, or drunk as an infusion by the native Thai and Malayans for a long time with opium-like effects and as an opium substitute when opium itself was unavailable or unaffordable. They are also claimed to be used as suppressor of the opiate withdrawal syndrome (Burkill, 1935). Large doses were claimed to result in vomiting, dizziness, and stupor. In Perak of Malaysia, pounded leaves are applied to wounds and whole, heated leaves over enlarged spleens (Marcan, 1929; Burkill, 1935). In Thai folkore medicine, Kratom leaves were also used for the treatment of diarrhoea. Various

Figure 1. Drawing of *Mitragyna speciosa* Korth.

parts of several species of Mitragyna have been used in local folklore medicine for a wide variety of diseases such as fever, colic, muscular pains, and for the expulsion of worms.

Kratom is illegal plants in Thailand since it was claimed to be addictted. In 1926, an extensive cultivation of Kratom in Thailand was recorded, with an increase of the marketing of the Kratom leaves (Burkill, 1935). Kratom leaves are much used for chewing by peasants, marketing gardeners, and labors in the central and southern regions of Thailand in order to endure great fatigue and exposure to heat so as to increase their work out-put. The Kratom leaves consumers can work in the rice fields, or perform other manual work without being exhausted from morning until evening even in a hot and sunny day. Addicts are, however, afraid of the rain which cause them to chill easily. Progression to Kratom leaves addiction is a gradual process of increasing in amount and frequency of the drug consumption. In 1975, a study of 30 Thai Kratom leaves users was reported (Suwanlert, 1975). Ninety percent chewed the fresh leaves or took it as a powder, adding table salt to prevent constipation followed by large volume of water. The leaves were chewed 3-10 times a day which stimulant effects beginning 5-10 minutes later. Side effects were listed as dry mouth, frequent micturition, constipation, small black faeces, anorexia and weight loss. The withdrawal syndrome included aggression, tearfulness, rhinorrhea, musculoskeletal aches and "jerky movement" (Suwanlert, 1975). The Kratom leaves used among Thai addicts belong to a variety with reddish midrib. Because of this harmful effects, the Government of Thailand passed a law (Kratom Act, B.E. 2486) which came into force on August 3, 1943 and by virtue of which it is forbidden to plant the tree, and the existing ones are to be cut down. Until now, Kratom is listed in the Schedule V under Narcotic Act, B.E. 2522. Thus Kratom is still an offence in Thailand either to possess any products of this plant

or to cultivate the trees. However, the measure chosen by the law to control Kratom leaves addiction has not been effective, since it is a local plant and the addiction does not have a bad reputation. The Kratom leaves consuming habit was noted to be culture bound to the Thais and largely a ritualistic, rural phenomenon, with village society accepting male addicts who work to support their families but not the female.

Mitragynine was first isolated by Field (1921) from *Mitragyna speciosa*. Mitragynine was found to a central nervous system stimulant rather than depressant (Grewal, 1932). The 1960's resurgence of interest had been spurred by a search for non-opiate analgesics. Macko et al. (1972) found mitragynine to be comparable with codeine as an analgesic and couch suppressant in dog, and that unlike codeine at equivalent doses, it did not cause emesis or dyspnoea. There was no opiate-like addiction syndrome, no antagonism by nalorphine, negligible anticholinergic action and minimal effect on gastric motility at analgesic levels. Furthermore, mitragynine had a little effect on the blood pressure of dogs, was only hypotensive in cats at high doses and was much less of a respiratory depressant than codeine. Cocaine-like effect was observed in five men observation who received mitragynine and 50 mg of pure mitragynine acetate produced nausea and vomiting in some subjects (Grewal, 1932). Chemically unrelated to any known analgesic, mitragynine also appeared to be significantly less toxic. There was no evidence of toxicity, such as tremor and convulsions observed after doses as high as 920 mg per 1 kg of body weight. Large doses in cats had stimulating effects qualitatively different from opiate "fear and rage" complex. Mitragynine was proposed to be metabolized to mitragynine pseudoindoxyl (Zarembo et al., 1974) that was 10 fold stronger than mitragynine in analgesic activity when administered by both oral and intraperitoneal routes to animals. It appeared that pre-clinical trial of mitragynine in human, carried out by

Smith, Kline and French Laboratories, reveal unacceptable side effect (Jansen and Prast, 1988)

Pain

Pain is characterized as an unpleasant sensation localized to a part of the body. It is a complicate response, often described in terms of a penetrating or tissue-destructive process (e.g., stabbing, burning, twisting, tearing, squeezing) and/or of a bodily or emotional reaction (e.g., terrifying, nauseating, sickening). Furthermore, anxiety and the urge to escape or terminate the feeling is asociated with moderate or higher intensity. These properties illustrate the duality of pain : It is both sensation and emotion. When it is acute, pain as well as behavioral arousal and a stress response, such as increased blood pressure, heart rate, pupil diameter, and plasma cortisol levels, are concomitant. In addition, local muscle contraction (e.g., limb flexion, abdominal wall rigidity) is often seen and may produce secondary tenderness (Isselbacher et al., 1994). The transmission of pain is now viewed not as a passive simple process using hard wired exclusive pathways but as messages arising from the interplay between neuronal systems, both excitatory and inhibitory, at many levels of the central nervous system which converge particularly on the spinal cord. One consequence of these processes is that, as has been long recognized from human experience and clinical data, there is not necessarily a clear relation between the stimulus and the response to pain in an individual. Since this plasticity in nociceptive systems can result from changes over short time courses the origins cannot be alterations in structure although these may occur over longer time courses. Recent research on the pharmacology of nociception has started to shed some well-needed light on these events which could have profound consequences for

the pharmacological treatment of pain (Dickenson, 1990 ; Dubner, 1991; McQuay and Dickenson, 1990).

The primary afferent nociceptor

A peripheral nerve consists of the axons of three different types of neurons: primary sensory afferents, motor neurons, and sympathetic postganglionic neurons. In the dorsal root ganglia in the vertebral foramina is a location of the cell bodies of primary afferents. The primary afferent axon bifurcates to send on process into the spinal cord and the other to innervate bodily tissues after emerging from its cell body. Primary afferents are catagolized by their diameter, degree of myelination, and conduction velocity. The largest-diameter fibers, A-beta, respond maximally to light touch and/or moving stimuli; they are present primarily in nerves that innervate the skin. In normal individuals, the activity of these fibers does not produce pain. There are two other classes of primary afferents: the small-diameter myelinated (A-delta) and the unmyelinated (C-fiber) axons . These fibers are present in the nerves to the skin and to deep somatic and visceral structures. Some tissues, such as the cornea, are innervated only by A-delta and C afferents. Most A-delta and C afferents respond maximally only to intense (painful) stimuli and produce pain when they are electrically stimulated; therefore, they are defined as primary afferent nociceptors (pain receptors). When A-delta and C axons are blocked, the ability to detect painful stimuli is completely abolished (Isselbacher et al., 1994).

At peripheral levels the majority of nociceptive signaling of thermal and mechanical pain arises from the activation of polymodal nociceptors which are innervated by C-fibers. The acute application of these modalities of stimuli results in a good relationship between the stimulus and the response. These types of stimuli form the basis for most experimental pain models both in man

and in animals (tail-flick, hot plate etc.). However, in the presence of tissue damage these fibers respond to local chemical stimulation and also become sensitized to chemical, thermal and mechanical stimuli. These alterations in sensitivity have been recognized for decades but only very recently has a central counterpart of this peripheral hyperalgesia been identified. The tissue damage can stimulate the synthesis of arachidonic acid metabolites from adjacent membranes, the cleavage of the precursor of bradykinin to release the active peptide and the release of peptides such as substance P and calcitonin gene-related peptide from the C-fibers via the axon reflex. This inflammatory soup, also containing 5-hydroxytryptamine (5-HT), K^+ ions and H^+ ions, activates and sensitizes the peripheral endings and causes vasodilatation and plasma extravasation so eliciting the swelling, pain and tenderness (Besson and Chaouch, 1987; Dray, 1991). Recently the advent of antagonists for bradykinin receptors has provided good evidence for the role of B_2 receptor subtype in these peripheral events with obvious implications for the genesis of new drugs (Haley et al., 1989). Another approach has been the use of capsaicin ,the pungent ingredient in peppers , which has remarkable selectivity for C-fibers. A high local dose of capsaicin first activates then inactivates peripheral C-fibers, a receptor-mediated event since the recently described analogue, capsazepine, competitively blocks this response (Dray, 1991). Other analogues of capsaicin which lack the pungency and depolarizing effects of capsaicin are being explored as potential analgesics, especially since one site of action of these capsaicin analogues may be blocking release at the central C-fiber terminals as well as the peripheral endings (Dray, 1991).

The spinal transmission of pain

Most of the neurotransmitters and their receptors found in the CNS are found in the spinal cord (Besson and Chaouch, 1987 ; Evans 1989). The transmitters are derived from either the afferent fibers, intrinsic neurons or descending fibers. The bulk of these are concentrated in the substantia gelatinosa, one of the densest neuronal areas in the CNS and crucial for the reception and modulation of nociceptive messages transmitted in via the peripheral fibers. C-fibers terminate in the outer lamina 1 and the underlying substantia gelationosa, whereas the large tactile fibers terminate in deeper laminae. However, in addition to the lamina 1 cells which send long ascending axons to the brain, deep dorsal horn cells also give rise to ascending axons and respond to C-fiber stimulation. In the case of these deep cells the C-fiber input may be relayed via interneurones or arrive on the dendrites of the cells which pass vertically into the gelatinosa. Considerable convergence and modulation of the responses of these deep cells can therefore result especially since inhibitory gelatinosa cells project towards the deep cells (Besson and Chaouch, 1987). Finally the reason for the good correspondence between pharmacological effects on the electrophysiology of these cells, behavioral tests in animals, psychophysical studies and reflex responses to pain is the likelihood that these deep sensory cells drive the withdrawal reflex and also give rise to ascending projections to areas of the brain involved in the perception of pain (Schouenborg and Sjolund, 1983; Schouenborg and Dickenson, 1985).

Central pathways for pain

The spinal cord and referred pain

From a clinical standpoint, the phenomenon of referred pain is underlied by the convergence of many sensory inputs to a single spinal pain-transmission

neuron. All spinal neurons receive input from the viscera and deep musculoskeletal structures as well as from the skin. The convergence patterns are determined by the dorsal root ganglion that supplies the afferent innervation of a structure. For example, the afferents that supply the central diaphragm are derived from the third and fourth cervical dorsal root ganglia. Primary afferents with cell bodies in these same ganglia supply the skin of the shoulder and lower neck. Thus sensory inputs from both the shoulder skin and the central diaphragm converge on pain-transmission neurons in the third and fourth cervical spinal segments. Because of this convergence and the fact that the spinal neurons are most often activated by inputs from the skin, activity evoked in spinal neurons by input from deep structures is mislocalized by the patient to a place that is roughly coextensive with the region of skin innervated by the same spinal segment. (Isselbacher et al., 1994).

Ascending pathways for pain

A majority of spinal neurons contacted by primary afferent nociceptors send their axons to the contralateral thalamus. These axons form the contralateral spinothalamic tract which lies in the anterolateral white matter of the spinal cord, the lateral edge of the medulla, and the lateral pons and midbrain. The spinothalamic pathway is crucial for pain sensation in humans. Interruption of this pathway produces permanent deficits in pain and temperature discrimination (Isselbacher et al., 1994).

The ventrobasal region of the thalamus is one major target area for spinothalamic tract axons. Spinothalamic tract axons connect to thalamic neurons that project to somatosensory cortex. This pathway from spinal cord to thalamus to somatosensory cortex appears to be particularly important for the sensory aspects of pain, i.e., its location, intensity, and quality. Spinothalamic tract axons

also connect to medial thalamic regions linked with the frontal cortex and the limbic system. This pathway is thought to subserve the affective or unpleasant emotional dimension of pain (Isselbacher et al., 1994).

Pain modulator

The pain produced by similar injuries is remarkably variable in different situations and in different people, and the expectation of pain has been demonstrated to induce pain without a noxious stimulus.

The powerful effect of expectation and other psychological variables on the perceived intensity of pain implies the existence of brain circuits that can modulate the activity of the pain-transmission pathways. Although there are probably several circuits that can modulate pain, only one has been studied extensively. This circuit has links in the hypothalamus, midbrain, and medulla, and it selectively controls spinal pain-transmission neurons through a descending pathway (Isselbacher et al., 1994).

The good evidence is that this pain-modulating circuit contributes to the pain-relieving effect of narcotic analgesic medications. Opioid receptors is contained in each of the component structures of the pathway and is sensitive to the direct application of opioid drugs. Furthermore, lesions of the system reduce the analgesic effect of systemically administered opioids such as morphine. Along with the opioid receptor, the component nuclei of this pain-modulation circuit contain endogenous opioid peptides such as the enkephalins and beta-endorphin (Isselbacher et al., 1994).

Prolonging pain and/or fear is the most reliable way to activate this endogenous opioid-mediated modulating system. That pain-relieving endogenous opioids are released following operative procedures and in patients given a placebo for pain relief has been shown (Isselbacher,et al., 1994).

Pain modulation is bidirectional. Pain-modulating circuits not only produce analgesia but are also capable of increasing pain. Both pain-inhibiting and pain-facilitation neurons in the medulla project to and control spinal pain-transmission neurons. Since pain-transmission neurons can be activated by modulatory neurons, it is theoretically possible to generate a pain signal with no peripheral noxious stimulus. Some such mechanism could account for the finding that pain can be induced by suggestion alone and may provide a framework for understanding how psychological factors can contribute to chronic pain (Isselbacher et al., 1994).

Central hyperalgesia

Obviously, the candidate systems for the substrates for the induction and the maintainance of hypersensitivity in the spinal cord will either be excitatory systems or the effects will result from the lifting of or interference with inhibitory controls.

Peptides

The roles of peptides such as substance P and the neurokinins, somatostatin, vasoactive intestinal polypeptide and others in excitatory nociceptive processing has been confounded by the lack of any specific or clear-cut effect on nociceptive events of antagonists, which are as yet only available for a limited number of these peptides anyway (Evans, 1989). The possible reasons may include simple a lack of efficacy, multiple receptor subtypes so that block of one receptor sub-type is insufficient to alter transmission, complex bidirectional roles of these agents in nociception or that peptides are not the primary nociceptive transmitters.

Excitatory amino acids

The recent finding of considerable numbers of peripheral sensory fibers containing glutamate and subsequently aspartate and the observation that 90% of substance P-containing fibers also contained glutamate is of interest (Battaglia and Rustioni, 1988). This co-existence would make it highly likely that, in addition to any glutamate released from intrinsic neurons, a noxious stimulus would induce a release of both peptides and excitatory amino acids from the afferent fibers. Interest has centered on the involvement of excitatory amino acids particularity actions via the N-methyl-D-aspartate (NMDA) receptor complex in the post-synaptic events in the spinal cord (Dickenson, 1990; Headley and Grillner, 1990). The NMDA receptor and its associated channel form a unique complex. Activation of the complex requires not only the binding of glutamate/aspartate to the receptor but glycine binding to an adjacent site as a co-agonist and a membrane depolarization to remove the resting Mg^{2+} block of the channel. The receptor complex is thus both ligand and voltage gated (Lodge and Johnson, 1990). There is good evidence for an involvement of this receptor in long-term potentiation in the hippocampus (Bliss and Lynch, 1988), in synaptic plasticity in the visual cortex (Collingridge and Lester, 1989) and in rhythmic activity in motor systems (Headley and Grillner, 1990).

Two original findings formed the basis for the subsequent studies which have addressed the problem of central spinal hypersensitivity. The first was the conditioning stimuli applied to activate C-fibers could result in a marked and prolonged increase in the flexion withdrawal reflex in spinal-decerebrate rats. (Woolf, 1983) The second was that the repetition of a constant intensity C-fiber stimulus could induce the phenomenon of wind-up whereby the responses of certain dorsal horn neurons increased dramatically despite the lack of change in

the input into the spinal cord (Mendell, 1966; Davies and Lodge, 1987; Dickenson and Sullivan, 1987; Dickenson and Sullivan, 1990). Wind-up is frequency dependent, and can augment responses of dorsal horn neurons by up to 20-fold in amplitude and convert 30 sec of stimulation to several minutes of response even after the cessation of the peripheral input (Mendell, 1966; Dickenson and Sullivan, 1987; Dickenson and Sullivan, 1990). Thus there are many similarities between wind-up and long-term potentiation in the hippocampus although the latter is considerably more prolonged, (Bliss and Lynch, 1988). Crucially, wind-up was shown to be sensitive to NMDA receptor antagonists such as 5-amino-phosphonovaleric acid, to channel blockers such as dizacilpine (MK-801) and ketamine and to antagonism of the associated glycine site (Davies and Lodge, 1987; Dickenson and Sullivan, 1987; Dickenson and Sullivan, 1990; Dickenson and Aydar, 1991). . Not surprisingly there are high levels of NMDA receptors in the spinal cord of a number of species including man (Shaw et al., 1991).

Serotonin and pain

Serotonin and Pain pathways in mammalian nervous system

At least four pathways exist in the mammalian central nervous system for relaying nociceptive information from peripheral "pain" receptors to the thalamus (Albe-Fessard et al., 1985; Besson and Chaouch, 1987). Of these four pathways the two most important for pain transmission in primates and rats appear to be the lateral spinothalamic tract and the spinoreticulothalamic pathway (Albe-Fessard et al.,1985; Peschanski and Besson, 1984). As indicated in figure 2, 5-HT

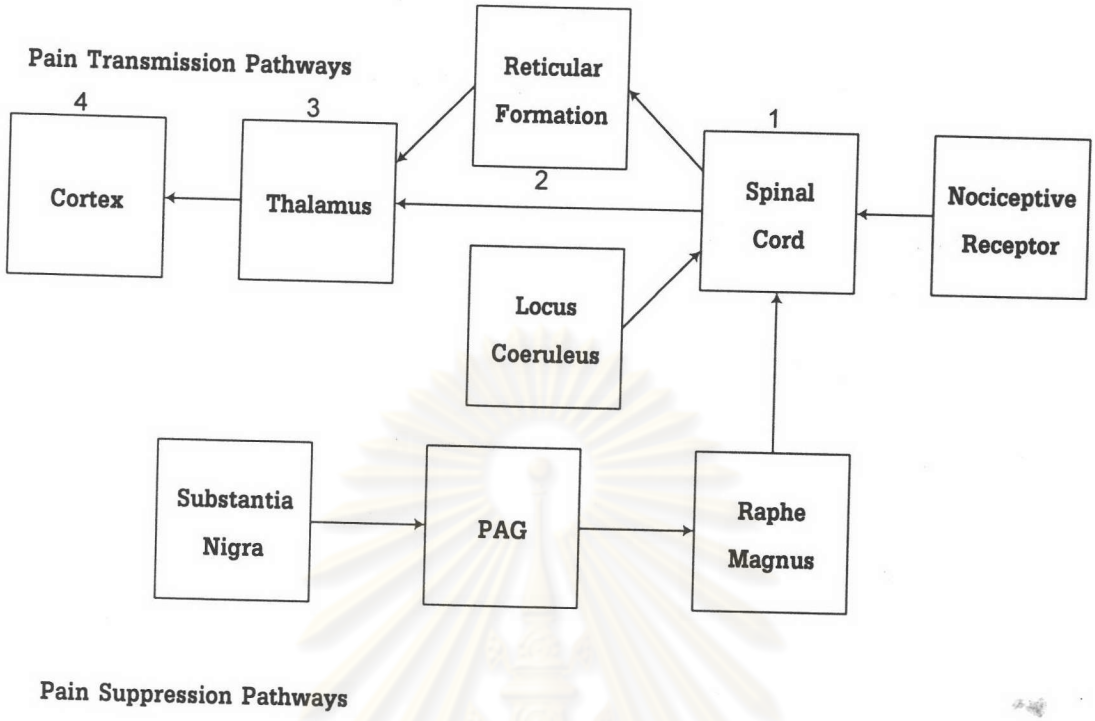

Figure 2 . Schematic diagram illustrating the anatomical organization of pain transmission and pain suppression pathways. The pain transmission pathways originate at a peripheral receptor and terminate in the cerebral cortex. The pain suppression pathways typically arise from brain stem nuclei and modulate the pain transmission pathways at the level of the spinal cord. For simplicity only four nuclei of the pain suppression are illustrated. Ascending serotonin pathways may moderate nociception by acting on components of either the pain transmission or the pain suppression pathways.

modulation of these two pathways could occur at several locations. Serotonin could modulate nociceptive input; (1) at the level of the spinothalamic or spinoreticular neurons in the spinal cord; (2) at the level of reticulothalamic neurons in the brain stem; (3) at the level of thalamocortical neurons in the thalamus or (4) at the level of the somatosensory cortex. The majority of studies to date have focused on modulation of nociceptive input at the level of the spinal cord by both 5-HT and non 5-HT descending systems (Basbaum and Fields, 1984; Fields and Besson, 1988). With regard to the origin of this descending 5-HT modulatory system, the spinal cord dorsal horn receives a dense serotonergic innervation from the 5-HT neurons in the raphe magnus, nucleus gigantocellularis pars alpha and other raphe nuclei of the lower brain stem (Dahlstrom and Fuxe, 1974). These 5-HT fibers course in the dorsal portion of the dorsolateral funiculus (Bullitt and Light, 1989) and appear to play an important role in the modulation of incoming pain sensation to the spinal cord (Basbaum, 1981; Mohrland and Gebhart, 1980; Rivot et al., 1980). Although the majority of studies have focused on descending 5-HT systems in endogenous pain control, there is evidence to suggest that ascending 5-HT pathways also play a role in the modulation of nociceptive transmission (Hunskaar et al., 1986, Yaksh, 1979). These ascending 5-HT pathways may modulate nociception at the level of the thalamus, at the level of the somatosensory cortex and/or at the level of the brain stem reticular formation.

5-HT modulation of nociception at the level of the thalamus

Thalamic Nuclei Involved With Pain Sensation

There are four thalamic nuclear groups that are prime candidates for an involvement in pain mechanisms, based on either their connections with ascending nociceptive pathways or clinical observation (Albe-Fessard et al.,

1985). These four thalamic nuclear groups include: (1) the ventrobasal complex, which includes the ventral posterior lateral and ventral posterior medial nuclei; (2) the medial part of the posterior nuclear complex; (3) the intralaminar complex, especially the central lateral nucleus; and (4) the nucleus submedius (gelationosus thalamic nucleus). In both primates and rats the ventrobasal complex receives a somatotopically organized projection form the spinothalamic tract (Albe-Fessard et al., 1985; Besson and Chaouch, 1987; Boivie, 1979). As eluded to above, the spinothalamic tract is involved in the transmission of nociception and pain (Besson and Chaouch, 1987) and the fact that it terminates in the ventrobasal complex of the thalamus suggests that this area is also involved in pain transmission. This concept has been supported by several studies in both rats and monkeys (Albe-Fessard et al., 1985; Guilbaud et al., 1980; Peschanski et al., 1980). It should be noted, however, that many neurons in the ventrobasal complex respond to contralateral non-noxious stimuli (Albe-Fessard et al., 1985). Despite the involvement of this nuclear group in mediating other types of somatic sensation, the ventrobasal complex receives inputs from nociceptive units with small receptive fields and in likely to encode discriminative aspects of nociception.

The posterior nuclear group is a relatively large, ill-defined region containing small cells situated dorsomedial to the ventrobasal complex in the rat (Faull and Mehler, 1985). In primates, as well as the cat, projections from the spinothalamic tract to the various zones of the nuclei of the posterior thalamus have been described (Albe-Fessard et al., 1985). Terminals from spinothalamic neurons are particularly dense in the medial division of the posterior nucleus and in the magnocellular zone of the medial geniculate body. The response properties of individual neurons in the posterior group was described (Poggio and Mountcastle, 1960) and found that most cells (71/123) required noxious intensities

of stimulation of the skin or deep tissue for their activation. Subsequent work by Guilbaud et al., 1980) found 40 out of 75 cells were activated exclusively by nociceptive stimuli while the remaining 35 neurons were excited by both innocuous and noxious stimuli. They concluded , in agreement with Poggio and Mountcastle that the posterior thalamic nuclei are likely to be involved in pain processing. Studies to date suggest that the posterior nuclei have something to do with defining a nociceptive stimulus as painful (Albe-Fessard et al., 1985).

The intralaminar complex of the thalamus includes several nuclei with diverse connectivity and presumably equally diverse functions (Albe-Fessard et al., 1985). The three intralaminar nuclei that have been implicated in pain mechanisms are the centre median nucleus, the central lateral nucleus and the parafascicular nucleus. Spinothalamic and trigeminothalamic fiber degeneration studies with silver impregnation methods first indicated that these ascending intralaminar fiber connections were restricted to the parafascicular and central lateral nuclei (Lund and Webster, 1967; Menetrey et al., 1980). More recent studies utilizing anterograde transport of WGA-HRP suggest that spinal projections to the intralaminar nuclei also distribute to the centre median nucleus (Mantyh, 1983; Peschanski and Ralston, 1985). However, labeling of the parafascicular and centre median nuclei in these studies has been attributed to transneuronal transport of the tracer via spinoreticulothalamic projections rather than direct transport via the spinothalamic tract (Peschanski and Ralston, 1985). Thus only the central lateral nucleus appears to receive direct spinothalamic projections.

Regarding the physiology of neurons in the intralaminar complex, a partially transected spinal cord was recorded from 6 intralaminar neurons in the study of monkeys (Perl and Whitlock, 1961). The cells could be excited by pinching the skin and following noxious stimulation they exhibited a prolonged

after-discharge. The responses of single units in the centre median-parafascicular complex to pin prick and noxious heat in conscious human patients during stereotaxic surgery has been recorded (Ishijima et al., 1975). The receptive fields were very large, occupying much of the body surface. Finally, nociceptive neurons have been found in the central lateral nucleus in rats and rabbits (Beitz, 1990). Like the nociceptive responsive neurons described in the monkey, these cells displayed a characteristic long-lasting after discharge following termination of the noxious stimulus. Since the central lateral nucleus receives spinothalamic tract input from ventral horn cells (Steven et al., 1989) and projects diffusely throughout the cortex, including the motor cortex (Aldes, 1988), this implies involvement in motor as well as general arousal responses to nociceptive inputs.

The nucleus submedius is a slender, elongated nucleus that lies in the ventromedial region of the thalamus (Faull and Mehler, 1985). A comparative study in the rat, cat and monkey has revealed that this nucleus receives a topographically organized projection from the spinal cord and caudal subnucleus of the spinal trigeminal nucleus (Craig, 1987). In the cat these ascending projections arise almost exclusively from layer 1 cells of the spinal and medullary dorsal horns. Based on the large receptive fields of its units and on its reciprocal connections to the forebrain, the nucleus submedius is believed to encode the affective aspects of nociception (Craig and Burton, 1981).

Localization of 5-HT immunoreactive fibers and 5-HT receptors in the thalamus

Both 5-HT immunoreactive fibers and 5-HT receptors have been described in the above four thalamic nuclear groups involved with pain sensation, but the density of 5-HT innervation and 5-HT binding sites varies considerably among these four regions. Although the distribution of serotonin within the thalamus has been described by several investigators (Chan-Palay,

1977; Peschanski and Besson, 1984; Steinbusch, 1981), the immunocytochemical data reported by Cropper et al. (1984) and Steinbusch (1981) seem most complete. Based on their immunocytochemical descriptions, the heaviest 5-HT innervation of the four nuclei implicate in pain sensation is found in the intralaminar nuclei including the central lateral , the centre median and the parafascicular nuclei. Moderate 5-HT innervation is observed in the posterior nuclear complex, while few serotonergic fibers are evident in the ventrobasal complex and the nucleus submedius. Consistent with the immunocytochemical data, receptor binding studies have shown the 5-HT binding sites are high in the intralaminar nuclear complex, but low in the ventrobasal complex (Biegon et al., 1989; Pazos and Palacios, 1985). Unfortunately a complete map of 5-HT binding sites in the thalamus is lacking and data regarding binding in the posterior nuclear complex and the nucleus submedius are unavailable. Based on the above findings one would predict that the greatest involvement of ascending 5-HT projections in pain modulation at the level of the thalamus would occur in the intralaminar nuclei and the posterior nuclear group.

Origin of 5-HT input to the thalamus and effects on thalamic neurons that respond to noxious stimuli.

The nucleus raphe magnus and adjacent nucleus gigantocellularis pars alpha supply input to the central lateral intralaminar nucleus and the nucleus submedius (Peschanski and Besson, 1984). The central lateral nucleus also receives input from the raphe pontis, while the nucleus submedius receives additional raphe projections from the raphe medianus (Bobillier et al., 1976; Peschanski and Besson, 1984). The raphe pontis and the raphe dorsalis provide fibers to the parafasicular nucleus. The ventrobasal complex receives input only from the raphe medianis (Bobillier et al., 1976; Peschanski and Besson, 1984).

Thus the thalamic nuclei involved in processing nociceptive information receive input from four different raphe nuclei. It is not clear at the present time what percentage of these raphe-thalamic projections are in fact serotonergic, since the raphe nuclei contain significant numbers of nonserotonergic neurons (Steinbusch and Nieuwenhuys, 1983).

Electrophysiological studies have shown that 5-HT applied micro-iontophoretically in the thalamus reduces thalamic neuronal responses to noxious stimulation (Anderson and Dafny, 1982) and that electrical stimulation of the dorsal raphe nucleus inhibits the responses of neurons in the nucleus parafascicularis to noxious stimuli via both ascending and descending pathways (Anderson and Dafny, 1983; Qiao and Dafny, 1988). After depletion of serotonin, dorsal raphe stimulation no longer produces inhibition but rather causes facilitation of discharges of parafascicular neurons (Anderson and Dafny, 1983). These studies suggest that the inhibition of parafascicular neurons by stimulation in the dorsal raphe is due to the release of 5-HT at the level of the thalamus. These results are also consistent with a recent glucose utilization study which demonstrated a statistically significant increase in glucose utilization in the parafascicular nucleus following electrical stimulation of the raphe dorsalis but not raphe medianis (Cudennec et al., 1988). It is interesting that stimulation of the raphe dorsalis also caused a significant increase in glucose utilization in the ventrobasal complex while stimulation of the raphe medianis did not. These results are contradictory to the anatomical data mentioned above which indicate a projection to the ventrobasal complex from the raphe medianis but not the raphe dorsalis.

Although a rigorous physiological analysis of the effects of 5-HT or raphe stimulation on the posterior nuclear group, the nucleus submedius and the other components of the intralaminar complex needs to be performed, the studies

reviewed above concerning the effects of 5-HT on nociceptive neurons in the parafascicular nucleus strongly suggest that 5-HT modulation of pain transmission at the level of the thalamus is likely. It is interesting that microinjection of morphine into the region of the nucleus submedius has been reported to produce analgesia as measured by the tail-flick method (Parolaro et al., 1988) further supporting a role for thalamic nuclei in pain modulation. It was also demonstrated that microinjection of morphine into the region of the somatosensory cortex caused a slight analgesic effect (Parolaro et al., 1988). The results suggest that pain modulation may also occur at the level of the cortex. Since the somatosensory cortex receives an organized projection from the ventrobasal complex and the posterior nuclear group (Spreafico et al., 1987), it is likely that nociceptive information is relayed to this cortical area from these nuclei and it is possible that ascending 5-HT systems can affect nociception processing within this cortical region.

5-HT modulation of nociception at the level of the somatosensory cortex

Localization of 5-HT immunoreactive fibers and 5-HT receptors in the somatosensory cortex.

The serotonergic fibers that innervate the cerebral neocortex arise from raphe dorsalis and raphe medianis (Anden et al., 1966; Bobillier et al., 1976; Parent et al., 1981). Although 5-HT fibers are distributed relatively uniformly throughout all six layers of the somatosensory cortex (Blue et al., 1988; Chan-Palay, 1977; Lidov et al., 1980; Steinbusch, 1981), they exhibit a differential orientation among the various layers (Lidov et al., 1980). For example, 5-HT axons are oriented parallel to the pial surface in layer I, while they exhibit a radial orientation in layers II and III. A recent ultrastructural examination of 5-HT immunoreactive fibers and terminals in the somatosensory cortex revealed that 5-

HT terminals rarely formed morphologically identifiable synaptic contacts and suggests that 5-HT may be released at points of close contact that lack membrane specializations (DeFelipe and Jones, 1988). A similar arrangement is found in the periaqueductal gray as described below. This absence of overt contacts and the possibility that only certain cells possess the corresponding receptors still makes it possible that there could be a selective indoleamine influence on particular cortical neurons.

It is interesting that there is a unique but transient pattern of 5-HT innervation of the somatosensory cortex during postnatal development such that there is a predominant localization of 5-HT terminals in layer IV and an aggregation of terminals in discrete groups giving a barrel-like appearance (Fujimiya et al., 1986). With regard to 5-HT receptors in the somatosensory cortex, the highest densities of 5-HT binding sites are found in layers IV and V and the lowest levels occur in layers II and III (Biegon et al., 1989; Pazos and Palacios, 1985). Layers I and VI exhibit intermediate levels of 5-HT binding sites. The somatosensory cortex appears to contain all three 5-HT receptor subtypes (5-HT_1, 5-HT_2 and 5-HT_3) (Nazarali et al., 1989; Pazos and Palacios, 1985). but 5-HT_2 receptors are especially prominent in layer IV (Bobillier et al., 1976; Peroutka, 1988).

Effects of 5-HT on the somatosensory cortex

Samanin et al. (1972) originally demonstrated that electrical stimulation of the raphe medianis in rats produced a decrease in peripherally evoked potentials in the primary somatosensory cortex. These investigators postulated that this effects was due to a release of serotonin. Consistent with this hypothesis is a subsequent study (Rivot et al., 1983) demonstrating that raphe stimulation does in fact cause the release of 5-hydroxyindoles in the

somatosensory cortex as measured by differential pulse voltammetry and that this release is ineffective when 5-HT synthesis is blocked by p-chlorophenylalanine. Iontophoretically applied 5-HT has been shown to inhibit the spontaneous activity of the majority of cortical neurons tested (Reader et al., 1979) and cortical neurons show a pronounced supersensitivity to iontophoretically applied 5-HT following selective lesioning of serotonergic fiber systems with 5,7-dihydroxytryptamine (Olpe et al., 1981). Furthermore, stimulation of the raphe nuclei has been shown to alter the spontaneous activity of cortical neurons (Jones, 1982). Electrical stimulation of the median raphe causes a significant but selective increase in glucose utilization in layer IV of the somatosensory cortex, while stimulation of raphe dorsalis causes a statistically significant increase in glucose utilization in layers II, IV and V (Cudennec et al., 1988). Surprisingly these cortical metabolic effects of raphe stimulation mimic the pattern of 5-HT innervation found during ontogeny of the somatosensory cortex. In sum these data suggest that the somatosensory cortex is under an inhibitory influence from the raphe medianis and dorsalis and it is probable that nociceptive input to the somatosensory cortex is influenced by this 5-HT system.

Possible 5-HT modulation of nociception at the level of the reticular formation

Several lines of evidence suggest that the brain stem reticular formation is involved in the phenomena of nociception (Besson and Chaouch, 1987; Casey, 1980; Fields et al., 1975; Menetrey et al., 1980), but data concerning the spinoreticulothalamic tract are much more difficult to interpret than data on the spinothalamic tract. Two major areas of the reticular formation, the bulbopontine zone and the mesencepthalic reticular formation, appear to receive spinal input and comprise the main termination areas of the spinoreticular tracts (Besson and Chaouch, 1987). The gigantocellular reticular nucleus (Gi) will be focused, since it

receives a significant spinal input (Albe-Fessard et al., 1974) and projects heavily to the intralaminar nuclei of the thalamus (Peschanski and Besson, 1984; Vertes et al., 1986). Spinoreticular neurons which project to the Gi are located principally in laminae VII and VIII of the spinal cord ventral horn with a few neurons also found in laminae V of the dorsal horn (Kevetter and Willis, 1982; Menetrey et al., 1980). Many neurons in these three laminae project to both the Gi and the thalamus (Kevetter and Willis, 1982) and it is interesting that a subset of these cells appears to contain enkephalin-like immunoreactivity (Nahin and Micevych, 1986). Neurons in the Gi respond to natural noxious stimulation of the face and body (Burton, 1968; Casey, 1969) as well as noxious visceral stimulation (Blair, 1985; Pavlasek et al., 1977). Since the Gi is a key nucleus in the spinoreticulothalamic pathway (Peschanski and Besson, 1984), it is conceivable that 5-HT projections may influence nociception by acting at the level of the Gi.

The Gi receives input from the nuclei raphe medianis, magnus and pallidus (Bobillier et al., 1976) and contains a relatively moderate density of 5-HT fibers (Chan-Palay, 1977; Steinbusch, 1981). This region also appears to have a moderate density of serotonin binding sites. An earlier study by Nakamura (1975) demonstrated that stimulation of the raphe medianis had an inhibitory effect on brain stem reticular neurons. However, a subsequent study indicated that both bulbar raphe stimulation and iontophoretically applied 5-HT excited neurons in the Gi (Briggs, 1977). It is likely, however, that 5-HT leads to excitation in the Gi by inhibition of a system that exerts a tonic inhibitory influence on neurons in this reticular nucleus, as has been proposed for 5-HT effects in the raphe magnus (Aimone and Gebhart, 1988). Although a major part of the 5-HT projection to the Gi is from descending 5-HT pathways rather than ascending systems, studies of the effect of 5-HT on reticulothalamic neurons that relay nociceptive information are lacking and this represents an important avenue of research.

Possible 5-HT modulation of nociception via effects on component of the endogenous pain suppress system

The majority of studies to date have focused on 5-HT modulation of pain transmission pathways. However, it seems feasible that 5-HT may also affect nociception by regulating the descending pain suppression system. Evidence to support such a hypothesis comes from the work which demonstrated that subcutaneous administration of the 5-HT_2 antagonist, ritanserin, acts supraspinally to activate pain modulating descending serotonergic, noradrenergic, dopaminergic and possibly opioid pathways (Barber et al., 1989). In addition the work of Nencini et al. (1988), showing that repeated administration of methylenedioxymethamphetamine causes an enhancement of morphine analgesia, suggests an effect on supraspinal 5-HT systems. Further evidence indication that 5-HT can affect components of the endogenous pain suppression system comes from studies on the nucleus raphe magnus. Llewelyn et al. (1983) have shown that 5-HT applied to the raphe magnus can elevate nociceptive thresholds and that microinjection of fenfluramine, which causes the release of 5-HT from synaptic terminals, also raises the threshold for nociception (Llewelyn et al., 1984). Dickenson and Goldsmith (1986) have subsequently provided further evidence for the role of serotonergic mechanisms in the control of nociceptive transmission by the raphe magnus, by demonstrating that injection of metergoline caused a reduction in the magnitude of neuronal responses to noxious stimuli. Unfortunately, the mechanisms of the effects of 5-HT in the raphe magnus are unclear since iontophoretic 5-HT has been reported to produce excitation or inhibition of raphe magnus neurons, some of which were raphespinal (Besson and Chaouch, 1987). Finally, it is of interest that systemic morphine administration causes a very significant increase in the 5-hydroxyindole

peak in the nucleus raphe magnus as measured by differential pulse voltammetry (Rivot et al., 1988), suggesting that 5-HT is released in this nucleus in response to morphine administration. In sum, these studies suggest that 5-HT acts on neurons in addition to having a major spinal effect. Other brain stem areas that have been implicated in descending analgesic mechanisms have a high density of 5-HT fibers and receptors and may also be modulated by this indoleamine. Three of these brain stem regions are discussed below.

Possible modulation of nociception at the level of the midbrain periaqueductal gray.

One region that has clearly been implicated as a key component of the endogenous pain suppression system is the midbrain periaqueductal gray (PAG) (Anderzik and Beitz, 1985; Basbaum and Fields, 1984; Besson and Chaouch, 1987; Fields and Besson, 1988). Both chemical (opiates and excitatory amino acids) and electrical stimulation of this midbrain region have been shown to produce a profound analgesia (Aimone and Gebhart, 1986; Behbehani and Fields, 1979; Besson and Chaouch, 1987). The PAG has a high density of innervation by 5-HT fibers (Clements et al., 1985; Steinbusch, 1981) that originate from all of the raphe nuclei (Beitz et al., 1986; Bobillier et al., 1976) with the raphe dorsalis providing the overall largest input. This is consistent with a recent glucose utilization study demonstrating that stimulation of the nucleus raphe dorsalis caused a statistically significant increase in glucose utilization in the PAG, while stimulation of the raphe medianis did not (Cudennec et al., 1988). Studies of 5-HT receptors have shown that the PAG has one of the highest densities of 5-HT binding sites in the midbrain. Thus it would seem likely that many neurons in this region would be affected by 5-HT. However, it should be noted that electron microscopic examination of 5-HT immunoreactive terminals in the PAG revealed

the presence of many 5-HT containing terminals but very few of these terminals actually formed synaptic contacts (Clements et al, 1985), suggesting that 5-HT is released into the PAG and must diffuse to its site of action. Although 5-HT has been shown to have an antiaversive role in the dorsal PAG (Graeff et al., 1986; Schutz et al., 1985) its effect on PAG neurons involved in descending analgesic mechanisms has not been evaluated. This is surprising in view of the dense 5-HT innervation of this area. It is interesting, however, that a recent study by Nichols et al. (1989) has demonstrated that stimulation produced analgesia elicited from both dorsal and ventral PAG sites is reversed by the serotonin antagonist, methysergide. It was not clear whether methysergide had its effect locally within the PAG or on descending 5-HT pathways.

In addition to its descending projections to other brain stem nuclei involved in analgesic mechanisms, the PAG projects to the parafascicular nucleus and ventrobasal complex of the thalamus (Barbaresi et al., 1982; Barbaresi et al., 1982). Stimulation of the PAG alters the activity of thalamic nociceptive neurons (Emmers, 1979) and thus this midbrain region appears to have an ascending as well as a descending effect on the nociceptive system. The possible modulation of these ascending and descending PAG projections by 5-HT remains to be elucidated.

Possible 5-HT modulation of nociception via effects in the locus coeruleus.

Electrical stimulation of the locus coeruleus induces analgesia and inhibitory effects on spinal and trigeminal nociceptive transmission (Besson and Chaouch, 1987; Blue et al., 1988). The locus coeruleus receives a high density of 5-HT immunoreactive fibers (Steinbusch and Nieuwenhuys, 1983) which contact noradrenergic neurons in the nucleus (Pickel et al., 1977). Receptor binding studies have further shown a moderate density of 5-HT binding sites in the locus

coeruleus. Segal (1979) has demonstrated that cells in the locus coeruleus give an excitatory response to a noxious stimulus and that this excitatory response is blocked by electrical stimulation of the nucleus raphe dorsalis. This finding is consistent with a recent study showing that stimulation of the nucleus raphe dorsalis causes a statistically significant increase in glucose utilization in the locus coeruleus. These studies suggest that 5-HT is capable of modifying the excitability of neurons in the locus coeruleus that respond to noxious stimulation and thus 5-HT may affect the descending pain suppression system by modifying the output of this region in response to a nociceptive stimulus.

Possible 5-HT modulation of nociception via effects in the substantia nigra.

Although the substantia nigra is typically considered a component of the motor system, several lines of evidence implicate this region in antinociception. A large percentage of neurons in the substantia nigra responds to noxious peripheral stimulation (Barasi, 1979). Electrical stimulation of the substantia nigra produces analgesia (Jurna et al., 1978; Segal and Sandberg, 1977) as does intranigral injection of opiates and GABA receptor agonists (Baumeister et al., 1988; Baumeister et al., 1987). One study has even claimed that bilateral intranigral microinjection of the opiate antagonist, naloxone, suppresses the antinociceptive effects of systemically administered morphine on the tail flick and hot-plate tests in a dose related manner (Baumeister et al., 1988). In addition electrical stimulation of this region inhibits responses of dorsal horn neurons to noxious peripheral stimulation (Barnes et al., 1979). Although it is unlikely that the substantia nigra has direct effects on the spinal cord dorsal horn, it projects to several nuclei of the endogenous pain suppression system including the periaqueductal gray (Beitz, 1982) and thus may influence the spinal cord indirectly via these nuclei.

The substantia nigra receives a dense 5-HT innervation (Steinbusch, 1981) originating from the nuclei raphe dorsalis and medianis (Dray et al., 1976). This midbrain region also has one of the highest densities of 5-HT binding sites and the highest level of 5-HT_{1B} sites in the central nervous system (Biegon et al., 1989; Pazos and Palacios, 1985). Recent observations that 37% of the 5-HT_{1B} binding sites in the substantia nigra disappear after 5,7-dihydroxytryptamine lesions favors the idea that some of these binding sites are located on 5-HT terminals (Verge et al., 1986). The high density of 5-HT binding sites in the substantia nigra suggests that this indoleamine plays an important role in this midbrain region. Consistent with such a role, a recent local cerebral glucose utilization study (Cudennec et al., 1988) has demonstrated that the substantia nigra was one of only three brain regions that showed significant decreases in glucose utilization following combined electrolytic lesions of the nuclei raphe dorsalis and medianis. Stimulation of the raphe dorsalis, but not the raphe medianis, on the other hand, resulted in a highly significant increase in glucose utilization in the substantia nigra (Cudennec et al., 1988). If the substantia nigra does play a role in the brain's endogenous antinociceptive system as the above data suggest, then it is likely that ascending 5-HT pathways may moderate this activity. However, an exclusive role of 5-HT in the substantia nigra's involvement with the motor system cannot be ruled out.

Differential modulation of nociception by the various classes of 5-HT receptors at the spinal level

5-HT_1 subtypes.

The potential analgesic/hyperalgesic effects of drugs acting selectively on the 5-HT_{1A} and 5-HT_{1B} receptors in the spinal rat was explored by evaluating the changes from baseline in the receptive field areas of three nociceptive

withdrawal reflexes following noxious mechanical stimulation (Murphy and Zemlan, 1990). The changes induced by the 5-HT_{1A} agonists 8-OH-DPAT and buspirone suggested that the sensitivity to noxious pinches was increased by the stimulation of 5-HT_{1A} receptors. Conversely, those evoked by the 5-HT_{1B} agonists, m-CPP and TFMPP, were consistent with a decreased sensitivity to noxious stimulation. Similarly, it was also reported that the two latter drugs and the other substituted piperazine 5-HT_{1B} agonist, MK-212, have clearcut antinociceptive effects in intact squirrel monkeys (McKearney, 1989). Indeed, all three drugs significantly increased the intensity of electrical stimulation (applied to the tail) which was freely tolerated by animals in a shock-titration procedure. The non-selective antagonist methysergide, but not the 5-HT_2 antagonists, ketanserin and pirenperone, prevented this effect, further supporting its mediation through the stimulation of 5-HT_{1B} receptors (McKearney, 1989). These are indeed two typical illustrations of a general agreement regarding the potential antinociceptive properties of 5-HT_{1B} agonists , at least in animals.

In contrast, the problem is much more complex in the case of 5-HT_{1A} agonists, since there are some controversy about some pro-nociceptive properties of these drugs. For instance, a decreased nociceptive sensitivity after the systemic administration of the 5-HT_{1A} agonist 8-OH-DPAT, using the hot plate test in intact mice similar to that induced by the non-selective 5-HT agonist 5-MeO-N, N-di-Me-tryptamine, had been observed, (Eide et al., 1988; Eide and Hole, 1989). Furthermore, the effect of 8-OH-DPAT was significantly enhanced after an intracerebroventricular injection of the neurotoxin, 5,7-DHT, as expected from a resulting supersensitivity of postsynaptic 5-HT_1 (notably 5-HT_{1A}) receptors (Eide et al., 1988). Conversely, 5-HT_1 receptor downregulation due to repeated treatment with 5-MeO-N, N-di-Me-tryptamine reduced the 8-OH-DPAT-induced increase in hot plate response latency (Eide et al., 1988). Evidence for an

antinociceptive action of 8-OH-DPAT has also been noted that in treated mice an increase in the temperature at which hindpaw lick occurs using the hot plate test (Fasmer et al., 1986). Similarly, another 5-HT_{1A} agonist, buspirone, also increases the paw lick latency in rats in the hot plate test (Bragin, 1989).

Further studies using various routes of administration have shown that the effects of systemic 8-OH-DPAT on thermal pain sensitivity can be reproduced by the intrathecal injection of the drug, strongly suggesting that spinal 5-HT_{1A} receptors are involved in the 8-OH-DPAT-induced reduction in the behavioral responses to noxious thermal stimuli (Fasmer et al., 1986). Clearly, these receptors do not mediate the slight hypoalgesia due to 8-OH-DPAT in the formalin test (for assessing the sensitivity to noxious chemical stimulation) since this effect was significant after systemic or intracerebroventricular but not intrathecal injection of the 5-HT_{1A} agonist (Fasmer et al., 1986).

Surprisingly, 8-OH-DPAT does not exhibit any significant antinociceptive effect in the tail-flick test (Bourgoin et al., 1980; Eide and Tjolsen, 1988; Rodgers and Shephard, 1989), which also involves a thermal noxious stimulus. One possible explanation to this apparent discrepancy has been recently proposed that 8-OH-DPAT by itself elicits tail-flicks in the absence of any external (thermal) stimulation (Millan et al., 1989). Therefore, the tail-flick test is obviously unsuited for assessing any possible modulation of nociceptive responses by the 5-HT_{1A} agonist 8-OH-DPAT. However 5-HT antagonists such as methiothepin and metergoline which act notably on 5-HT_{1A} receptors give clearcut hyperalgesic in the tail-flick test (Proudfit and Hammond, 1981). Thus, it can reasonably be assume that, without the specific problem inherent to 8-OH-DPAT , this agonist would probably also exhibit analgesic properties in the test (Millan et al., 1989). Indeed using appropriate experimental conditions, 8-OH-DPAT-induced analgesia

with the tail-flick test in both rats and mice was successfully demonstrated (Archer et al., 1987).

In most cases where nociceptive sensitivity was assessed using a noxious thermal stimulation, 8-OH-DPAT and other 5-HT_{1A} agonists appeared to exert an analgesic effect. In contrast, an hyperalgesic effect of 8-OH-DPAT was noted using noxious mechanical stimuli (Murphy and Zemlan, 1990). This discrepancy further illustrates that the bulbo-spinal serotonergic system (possibly via serotonergic synapses functioning with 5-HT_{1A} receptors) probably belongs to a neural mechanism selectively involved in a negative modulation of pain due to noxious thermal (but not mechanical or chemical) stimuli (Kuraishi et al., 1985; Proudfit and Hammond, 1981).

On account of the presence of 5-HT_1 receptors on both presynaptic afferent fibers and postsynaptic 5-HT target cells, several hypotheses can be put forward regarding the cellular events which mediate the analgesic effects of 5-HT_1 agonists. Thus electrophysiological recordings have confirmed that both pre- and postsynaptic sites of action are probably involved in the analgesic effect of intrathecal 5-HT (Carsten et al., 1987; Headley et al., 1978). In reference to the modulatory effects of opioids on the release of neuropeptides (notably substance P and CGRP) from primary afferent fibers (Jessell and Iversen, 1977; Pohl et al., 1989), some control by 5-HT agonists acting on the receptors located on these fibers might also be expected on the same parameter. It has been shown that 5-HT itself does not affect the release of substance P-like material (SPLM) from slices of the dorsal zone of the spinal cord (Pang and Vasko, 1986). However more recent investigations revealed a significant modulation of CGRP release by the indoleamine suggesting that only a subpopulation of primary afferent fibers (those where CGRP is not co-localized with SP) might be endowed with 5-HT_1 receptors.

5-HT at 0.1 μM, but not at higher concentrations, significantly enhanced the K^+-evoked release of Met-enkephalin-like material (MELM), as expected from an excitatory influence of the indoleamine on enkephalinergic interneurons within the dorsal horn (Hamon et al., 1990). The fact that only a low concentration of 5-HT was active strongly suggests that the receptor mediating this effect has a high affinity for the indoleamine, i.e. belongs most probably to the 5-HT_1 class than to any other 5-HT receptor class . However, further studies with selective agonists of 5-HT_{1A} , 5-HT_{1B} , 5-HT_{1C} and even 5-HT_{1D} receptors will have to be performed to identify which receptor type controls MELM release within the dorsal horn.

On account of the presence of serotoninergic terminals making synaptic contacts with enkephalinergic interneurons (Glazzer and Basbaum, 1984), the stimulatory effect of 5-HT on MELM release might well be the result of a direct action of the indoleamine on 5-HT_1 receptors located on these interneurons. However, other mechanisms might also be involved through a 5-HT effect at any level of complex neuronal network in which enkephalinergic interneurons would be one link among many others. Indeed the bulbo-spinal noradrenergic system might also be included in this postulated network, since the analgesia triggered by 5-HT agonists such as 8-OH-DPAT, m-CPP and 5-MeO-N, N-di-Me-tryptamine can be markedly reduced by drugs blocking the central noradrenergic neurotransmission (Archer et al., 1987; Post et al., 1986; Yaksh and Stevens, 1988). Furthermore, intrathecal 5-HT- or quipazine-induced analgesia can be suppressed by selective spinal cord NA depletion (Post et al., 1986) indicating that the integrity of the descending noradrenergic system is required for spinally-mediated analgesia. Therefore the simplest network possible involved in the analgesic effect of intrathecal 5-HT might be composed of the descending noradrenergic system under the excitatory control by 5-HT, and projecting onto

enkephalinergic interneurons. Numerous questions to be answered arise from this simple model; for instance; are 5-HT receptors, notably of the 5-HT_1 class(es), located on nor-adrenergic terminals within the dorsal horn? Is NA exerting an excitatory influence on spinal enkephalinergic interneurons? However, even in the case of a positive answer to the latter question, it will still remain to be explained why neither the analgesic effect of intrathecally injected NA nor that of 5-HT via the same route can be antagonized by the opiate antagonist naloxone (Yaksh and Stevens, 1988). Perhaps naloxone which is essentially an antagonist at mu opioid receptors (Kosterlith, 1985), does not prevent the effects of enkephalins which are preferentially acting on delta opioid receptors (Kosterlith, 1985)? Further experiments with the presently available delta opioid antagonists should be performed in order to truly assess the involvement of an enkephalinergic link in the analgesic effect of 5-HT administered via the intrathecal route.

Another problem relevant to the analgesic effect of 5-HT_1 agonists acting at the spinal level concerns its possible physiological significance. In other words, is endogenous 5-HT able to trigger, like exogenous 5-HT_1 agonists, the spinal mechanisms responsible for behavioral analgesia? Indeed drugs such as d-fenfluramine, p-chloroamphetamine (Berge et al., 1985) or porcine calcitonin (Bourgoin et al., 1988) which release endogenous 5-HT at the spinal level when injected intrathecally, have been described to significantly enhance the nociceptive thresholds as measured in the hot plate and tail flick tests in rats (Berge et al., 1985; Spampinato et al., 1984). Furthermore, their effect can be prevented by 5-HT antagonists or previous destruction of descending serotoninergic systems by 5,7-DHT (Berge et al., 1985; Clementi et al., 1985). Interestingly, not only drugs but noxious stimuli and electrical stimulation of C fibers conveying the pain signals can also evoke 5-HT release at the spinal level (Hamon et al., 1988) Furthermore, a recent study shown that thermal noxious

stimuli (for instance dipping the tail in water at 52 °C) were more efficient in triggering spinal 5-HT release than mechanical (pinches) and chemical (subcutaneous injection of formalin) noxious stimuli in halothane-anaesthetized rats (unpublished observations). Since exogenously administered 5-HT_1 agonists appear to be especially potent in decreasing the sensitivity to thermal noxious stimuli (Kuraishi et al., 1985; Proudfit and Hammond, 1981; Schmauss et al., 1983), the observation that the latter stimuli were also especially efficient to activate descending bulbo-spinal serotoninergic projections may suggest that these neurons, in fact, participate in a physiological control of pain due to noxious thermal stimulation

If the above conclusion also applies to humans, one may understand why 5-HT_1 agonists are devoid of any analgesic effect in patients suffering form various pains, particularly since thermal noxious stimulation was not involved in the various reports published to date. For instance, neither the 5-HT_{1A} agonist, buspirone, nor the 5-HT_{1B} agonist, m-CPP, were able to relieve pain in post-herpetic neuralgia or neuropathy (Kishore-Kumar, 1989). Moreover, it has to be further emphasized that the negative results with m-CPP are not surprising since its natural targets, the 5-HT_{1B} receptors, are absent in the human CNS. Instead, 5-HT_{1D} receptors are present, and their affinity for m-CPP is much less than that of 5-HT_{1B} receptors in rodents (Hoyer et al., 1988). Therefore, further investigations regarding the possible analgesic effects of 5-HT_1 agonists in humans should consider drugs acting on 5-HT_{1D} instead of 5-HT_{1B} receptors. Interestingly, the only selective 5-HT_{1D} agonist which has been developed so far, sumatriptan has been recently using to stop migraine attacks , and it would be particularly interesting to examine whether this drug is also potent to relieve other forms of pain.

5-HT_2 subtypes.

Using the tail flick and writhing tests, significant antinociceptive effects of the 5-HT_2 antagonists, ritanserin and ketanserin, have been described in the rat (Barber et al., 1989). Furthermore, studies in humans have also demonstrated an analgesic action of these drugs in appropriate experimental tests (Sandrini et al., 1986). However as expected from the very low to non-detectable density of 5-HT_2 receptors within the dorsal horn (Monroe and Smith, 1983; Palacios and Dietl, 1988), these effects are not triggered at the spinal level, but more probably via the blockade of supraspinal 5-HT_2 receptors, leading indirectly to an activation of descending monoaminergic neurons (Barber et al., 1989). Indeed the intrathecal administration of ritanserin does not reproduce the effect of its systemic injection, but instead prevents it (Barber et al., 1989).

Further evidence for the involvement of 5-HT_2 receptors in pain control has been obtained by using the selective agonist DOI [1-(2, 5-dimethoxy-4-iodophenyl)-2-amino-propane] where an increased latency in the tail flick test was observed in pentobarbital anaesthetized mice treated with this drug (Banks et al., 1988). Therefore the present situation is confusing since both a 5-HT_2 agonist, DOI, and 5-HT_2 antagonists, ketanserin and ritanserin, exert the same effect, i.e. an apparent decrease in behavioral responses to noxious stimuli. It must be emphasized, however, that the receptors involved might be differentially located, and peripheral rather than central 5-HT_2 receptors probably participate in the analgesic effect of DOI. Indeed, evidence for an excitatory effect of 5-HT via 5-HT_2 receptors on C sensory fibers supplying the ankle joint has been recently obtained (Grubb et al., 1988). Nevertheless, a general agreement on the location of 5-HT_2 receptors involved in pain control has not been achieved. For instance, that spinal 5-HT_2 receptors mediate the analgesic effects of drugs such as DOI, quipazine and MK-212 via the intrathecal route in rats was proposed (Solomon

and Gebhart, 1988) . However, it must be pointed out that none of these drugs are really selective for 5-HT_2 receptors. Thus other receptor types at the spinal level might have been involved in the analgesic effect described by these authors, especially since they did not attempt to prevent this effect by selective 5-HT_2 antagonists.

The observation that the 5-HT_2 antagonist pirenperone exerts hyperalgesic effects, and together with ketanserin, attenuate analgesia induced by either morphine or stimulation of the nucleus raphe magnus (Paul et al., 1989) may be considered further indirect support for the participation of 5-HT_2 receptors in 5-HT-induced analgesia at the spinal level . However it must be pointed out that both pirenperone and ketanserin also exhibit α-adrenergic antagonist properties (Sanders-Bush, 1988) which might well account for their effects on nociception.

In conclusion, these data suggest that 5-HT_2 receptors probably participate in pain control. Although it can be reasonably excluded that these receptors are located at the spinal level, considerable work has yet to be performed to understand the mechanisms of nociceptive modulation by 5-HT_2 ligands.

5-HT_3 subtypes.

The availability of several potent and selective 5-HT_3 antagonists has allowed recent investigations on the possible involvement of central and/or peripheral 5-HT_3 receptors in pain modulation. Clearly the latter receptors are implicated in the pain caused by application of 5-HT to a blister base in man since very low doses of the selective 5-HT_3 antagonist ICS 205-930 administered locally can prevent the effect of the indoleamine (Richardson et al., 1985). Similarly, Eschalier et al. (1989) recently demonstrated that stimulating peripheral 5-HT_3 receptors is responsible for the hyperalgesia induced by the subcutaneous injection of carrageenan into a hindpaw in rats.

Systemic 5-HT_3 antagonists also possess analgesic properties in the formalin test but not in relevant thermal and mechanical nociceptive tests in rats (Giordano, 1989). Furthermore, the intracerebroventricular injection of these drugs was ineffective in all tests which confirms that the blockade of peripheral 5-HT_3 receptors entirely accounts for their analgesic effects (Giordano, 1989). However, intrathecal ICS 205-930 can block the increase in tail-flick and hot plate latencies due to intrathecal 5-HT in rats (Glaum et al., 1988). This finding is indeed quite surprising since several other groups have shown that 5-HT antagonists not acting on 5-HT_3 receptors, but preferentially on 5-HT_1 receptors (at the spinal level), also prevent the antinociceptive effect of intrathecal 5-HT (Schmauss et al., 1983). In addition, the attempts to uncover a spinal action of 5-HT_3 agonists (such as 2-Me-5-HT phenylbiguanide) and antagonists (ICS 205-930 ; and zacopride) gave negative results. Neither the release of SPLM nor that of MELM could be modulated by 5-HT_3 ligands either in vitro or in vivo. Although these negative findings might be explained by a rapid desensitization of 5-HT_3 receptors (Richardson et al., 1985), they address the question of the true function of spinal 5-HT_3 receptors, notably those located on capsaicin-sensitive primary afferent fibers (Hamon et al., 1989).

Different types of pain

Clinical pain associated with injury or disease is a complex phenomenon, which involves sensory, affective and cognitive processes (Livingstone, 1953; Melzack, 1961; Melzack and Casey, 1968), and is likely, therefore, to be at least partially mediated by levels of the central nervous system (CNS) higher than the brainstem (Melzack and Casey, 1968). However, research on pain in animals has concentrated on modification of the spinally-mediated sensory component, as indicated by the popularity of pain tests such as the tail flick test. This test

(D'Armour and Smith, 1941), which is the most commonly used pain test in animals, measures the withdrawal response to threshold-level thermal stimulation of the tail. The tail flick response occurs in animals transected at the spinal level indicating that it is a spinal reflex (Carroll and Lim, 1960; Irwin et al., 1951). Recently, Chan and Dallaire (1989) have demonstrated that the sensory component of experimental pain in humans is "set" at the spinal interneuronal level. This suggests that spinally-mediated reflex-withdrawal tests in animals primarily measure the sensory dimension of pain, and thus may be more accurately classified as tests of nociception.

There is reason to believe that there are other sub-types of pain that are mediated by higher levels of the neuraxis. Low intensity electrical stimulation of the tail produces a tail withdrawal response in rats while higher intensity stimulation produces a vocalization response (V) during stimulation, and if the stimulation intensity is increased the vocalization response persists after cessation of the stimulus (vocalization after-discharge - VA) (Carroll and Lim, 1960; Hoffmeister and Kroneberg, 1966). As mentioned above, the tail withdrawal response occurs in animals transected at the spinal level indicating that it is a spinal reflex. In contrast, V responses are eliminated by transection at the brainstem level but are not affected by more rostral lesions, while the VA response is eliminated by diencephalic lesions, and more caudal lesions, but is not affected by more rostral lesions. This suggests that V responses are mediated by the brainstem while the VA response is mediated at diencephalic levels (Carroll and Lim, 1960; Hofffmeister and Kroneberg, 1966; Paalzow and Paalzow, 1975).

Pain investigators have used many other types of test for which the level of the neuraxis mediating the responses either remains unknown or is believed to be supraspinal. In most of these pain tests the pain stimulus is more continuous,

and more severe, than in reflex-withdrawal tests. As indicated above, the vocalization (V) test is an example of a pain test in which the responses appear to be mediated supra-spinally at the level of the brainstem. The hot-plated test is another pain test in which responses are thought to involve an integrated escape response that may be mediated by supra-spinal mechanisms (Eddy et al. 1950; Eddy and Leimbach, 1953; Woolfe and McDonald, 1944). There are two pain tests for which there is evidence that the responses are forebrain mediated. One of these, the VA test, was described above, the other, the formalin test (Dubuisson and Dennis, 1977) is described below.

In the formalin test, pain and tissue damage are produced by a subcutaneous injection of dilute formalin into an animal's paw. The response in rats is relatively complex and involves favoring, lifting, and licking the injected paw. This test was designed to provide a model of clinical pain by subjecting the animal to conditions as similar, as ethically possible, to those that produce continuous pain in humans. A reviewer of pain testing methods in animals noted that "the special value of the improved formalin test (as a model of clinical pain) lies in the fact that the authors describe related subjective experience in people. Injection of a small amount of formalin into the index finger immediately produces "intense", sharp, stinging, burning pain which, after 5 min, gives way to a steady, throbbing ache that gradually disappears after 30-60 min, leaving only mild residual tenderness at the site of injection." (Hunskaar et al., 1985).

Forebrain structures appear to be involved in formalin test pain in rats since unilateral knife cuts through the medial forebrain bundle and the medial internal capsule and/or the thalamus reduce pain much more in the ipsilateral than in the contralateral forepaw, suggesting that forebrain structures ipsilateral to the formalin injection are involved in the elaboration of the pain produced by the injection (Amodei and Paxinos, 1980). Thus, there appear to be at least three

distinguishable sub-types of pain mechanisms which appear to be located at the spinal level, the brainstem level and the forebrain level of the CNS respectively. Spinal nociception may be assayed with pain tests such as the tail flick test which measure reflexive withdrawal responses, brainstem mediated pain may be assayed by measuring V responses, and perhaps the more complex integrated escape response in the hot plate test, while forebrain mediated pain may be assayed with the VA and formalin tests.

There is some evidence that clinical pain and the pain produced in the VA test may involve a significant affective component. In humans, opiates greatly reduce the affective quality of pain, but have less effect on its sensory quality (Beecher, 1959; Cushny, 1918; Jaffe and Martin, 1985). This is illustrated by the apparently paradoxical comment of a patient in a recent clinical morphine analgesia study: "I'm not in pain, it hurts" (Franklin et al., 1990). Morphine has also been shown to reduce the anxiety associated with the anticipation of severe pain (Hill et al., 1952). In rats, VA responses appear to have a greater affective component than V responses or tail withdrawal responses. Spectrographic patterns of VA are similar to those evoked by vocalizations which are conditioned emotional responses, whereas patterns of V do not resemble those evoked by conditioned emotional responses (Levine et al., 1984). Furthermore, VA responses are attenuated by the anxiolytic drug diazepam while V responses are not (Hoffmeister, 1966; Levine et al. 1984). In human studies diazepam has been reported to selectively alter the affective but not the sensory description of noxious electrocutaneous stimuli (Gracely et al., 1978), and to alter the emotional component of pain without altering sensory sensitivity (Chapman and Feather, 1973). Since the pain produced in the formalin test is continuous, moderately severe and relatively long-lasting it is likely that this type of pain also has a significant negative affective component.

Both mitragynine and serotonin are indole compounds (structures shown below). As reviewed, mitragynine is claimed to have antinociceptive action. In the light that serotonin is involved in modulation of pain, it is, therefore, pertinent to postulate a relationship between mitragynine and serotonin in mitragynine induced analgesia. In this study using some animal models, electrophysiology (microiontophoresis) and the measurement of neurotransmitters release, we try to find out the relationship between serotonin and mitragynine.

In this study the objectives are :

1. To study pharmacological action of mitragynine with special emphasis on analgesic action.

2. To elucidate the mechanism of action of mitragynine emphasising dopaminergic and serotonergic related activities including effects of mitragynine on single neurons.

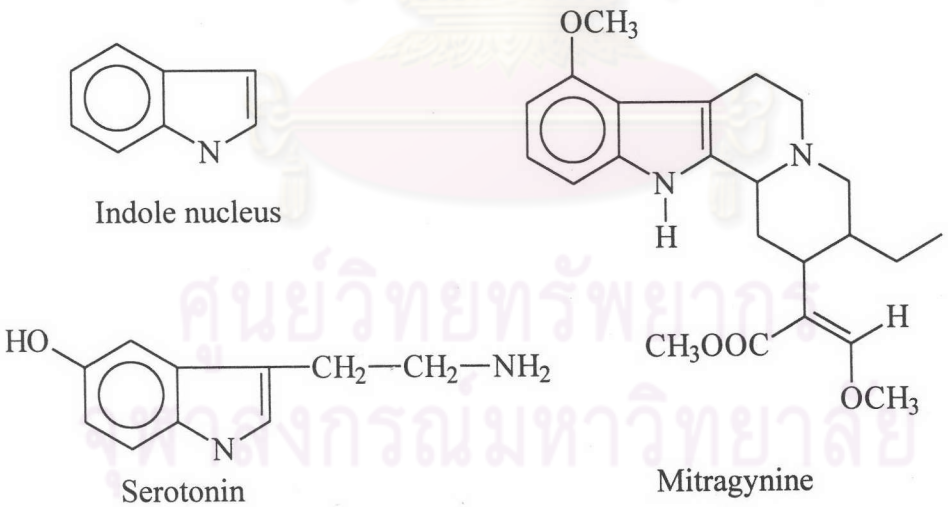

Figure 3. Structures of indole nucleus, serotonin and mitragynine