CHAPTER I

INTRODUCTION

Pain is a widespread clinical problem. Mechanism of pain perception is complcated and depends on interaction among various parts of central nervous system (CNS). The central sensory connection, which is the main ascending pathway that projects directly to the thalamus, subserves the sensations of pain, temperature and crude touch. Two types of afferent fibers have been identified: very fine unmyelinated c-fibers (0.4 to 1.1 μm in diameter) and thinly myelinated A delta (Aδ-fibers) (1 to 5 μm in diameter). The terminal receptors of these primary pain afferents are freely branching nerve ending. The c-fibers respond mainly to mechanical and thermal stimuli and Aδ-fibers respond to extremes of touch and pressure. The cell bodies of these afferent fibers lie in the dorsal root ganglion. The central axons pass via the dorsal root and synapse in the dorsal horn. The axons of these dorsal horn neuron decussate in the anterior spinal commissure, and ascend in the anterolateral fasciculus to brainstem and thalamic structures, mainly ventral posterior nuclei. Then, project to the postcentral cortex and to the secondary sensory cortex. This pain pathway is called spinothalamic tract (Figure 1).

Physiology of central pain modulation

Rapid progress in understanding the mechanism of pain modulation began with the serendipitous discovery of stimulation-produced analgesia in rats (Basbaum and Fields, 1984; Baskin et al., 1986; Fields and Basbuam, 1994). In human subjects, stimulation of the midbrain periaqueductal gray (PAG) matter through stereotactically implanted electrodes has also been shown to produce a state of analgesia (Renolds, 1969).

PAG is part of a circuit that controls nociceptive neurons in the dorsal horn. Its major outflow is caudally to the rostral ventromedial medulla (RVM). The RVM in turn projects massively and selectively to pain-transmitting neurons in the dorsal horn of the spinal cord. Electrical stimulation in the PAG or the RVM produces behavioral analgesia and inhibition of spinal transmission neurons (Besson and Chaouch, 1987).

The RVM, PAG and dorsolateral pontine tegmentum (DLPT) contain opioid receptors and dense concentration of endogenous opioid peptides; enkephalin. When opioid agonists such as morphine are microinjected into either the PAG or RVM, a powerful analgesic effect is produced (Fields et al., 1991). Furthermore, these brainstem sites are linked to each other by opioid synapses (Kiefel et al., 1993; Pan and Fields,1996; Roychowdhury and Fields, 1996). In considering nonopioid receptor contributions to nociceptive modulation, there is an extensive body of evidence implicating the role of a monoamine, serotonin (5-HT) (Basbuam and Fields, 1984; Yaksh, 1985; Le Bars, 1988; Sawynok, 1989). Supraspinal opioid analgesia is attenuated by the intrathecal administration of 5-HT antagonists (Barbaro et al., 1985). Spinal 5-HT is derived entirely from neurons in the brainstem, and this monoamine is

released within the spinal cord following antinociceptive brainstem stimulation (Hammond et al., 1985). Moreover, analgesia induced by electrical stimulation of the PAG in rats can be inhibited by injection of methysergide into nucleus raphe manus (NRM) (Roberts, 1984). Activation of descending bulbospinal serotoninergic neurons carried in the dorsolateral funiculus and release of 5-HT in the dorsal horn of the spinal cord. This results in inhibtion of segmental nociceptive input. In support of this is the observation that serotoninergic nerve fibers originating from PAG do innervate the NRM in rats (Beitz, 1982).

Several lines of existing evidences suggest the involvement of serotoninergic inhibitory pathway in mediating the analgesic action of morphine. In conscious rats with chronically implanted brain cannulae, the injection of morphine into NRM induces antinociception which can be antagonized by the concomitant administration of 5-HT antagonist cinanserin (Llewelyn et al., 1984). This finding suggested that morphine produces its analgesic effects through the local release of 5-HT. Direct support for this interpretation was provided by Pycock et al. (1981) who showed that both morphine and the stable enkephalin analogue, FK 33-824, release 5-HT from superfused slices of PAG and NRM (Pycock et al., 1981). Moreover, direct injection of morphine into the NRM also releases 5-HT in the spinal cord and produces antinociception (Shiomi et al., 1978; Yaksh et al., 1979; Vasko and Vogt; 1982), as does electrical stimulation of this nucleus. It thus appears that at least part of the antinociceptive action of morphine is mediated through activation of serotoninergic neurons in the brainstem.

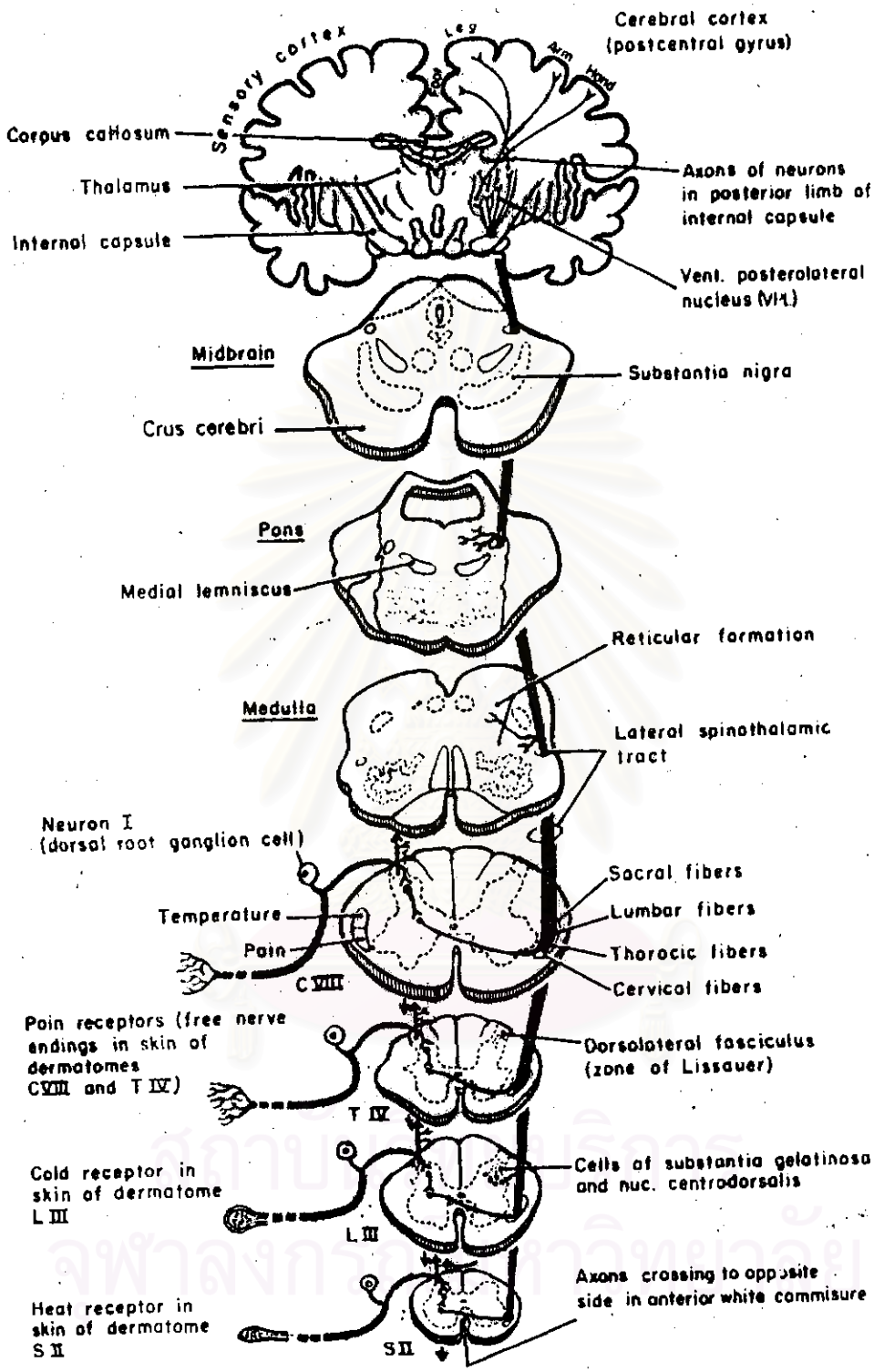

Figure 1. Schematic diagram of the lateral spinothalamic tract.

Anatomy of the dorsal horn

The spinal cord is divided into several broad anatomic regions, which are further divided on the basis of descriptive anatomy into several lamina (Rexed, 1954). In the nerve, large and small afferents are anatomically intermixed. As the root approaches the spinal cord, there is a tendency for the large myelinated afferents to move medially and to displace the small unmyelinated afferents laterally. Thus, although this pattern is not absolute, large and small afferent axon enter the dorsal horn via the medial and lateral aspects of the dorsal root entry zone, respectively. An appreciable number of unmyelinated afferent fibers that arise from dorsal root ganglion cells, however, also exist within the ventral roots, and these likely account for the pain reports evoked by ventral root stimulation in classic clinic studies.

Upon entering the spinal cord, the central processes of the afferents collateralize in two ways. First, the afferents send fibers rostrally and caudally up to several segments in the tract of Lissauer (small C fiber afferents) or into the dorsal columns (large afferents) and into the segment of entry. Second, upon penetration of the fibers into the parenchyma, the terminal fields also ramify rostrally and caudally for several segments.

Terminals from the small myelinated fibers are located in the marginal zone or lamina I of Rexed, the ventral portion of lamina II, and throughout lamina III. Fine-caliber, unmylinated fibers generally terminate throughout lamina II and in lamina X around the central canal (Figure 2).

Dorsal Horn Neurons

Though exceedingly complex, the second-order nociresponsive elements in the dorsal horn may be considered in four principal classes on the basis of their approximate anatomic location.

Marginal Zone (Lamina I)

The large neurons of the marginal zone are oriented transversely across the cap of the dorsal gray matter. Some project to the thalamus via contralateral ascending pathways, and others project intrasegmentally and intersegmentally along the dorsal and dorsolateral white matter. Population of these neurons respond to intense cutaneous and muscle stimulation.

Substantia Gelatinosa (Lamina II)

A significant proportion of the substantia gelatinosa neurons receive input from A-delta and C fibers and are frequently excited by activation of thermal receptive or mechanical nociceptive afferents. The properties of substantia gelatinosa neurons are not well understood, but unlike many spinal neurons, these cells exhibit complex response patterns with prolonged periods of excitation and inhibition following afferent activation.

Nucleus Proprius (Lamina III and IV)

Cells in the nucleus proprius may be broadly classed as those that respond almost uniquely to innocuous (A-beta) input, or low-threshold neurons, and those that response to A-beta, A-delta , and C input, or wide dynamic range (WDR) convergence neurons. The former class responds to brush or touch but shows no elevation in activity with prolonged pinch.

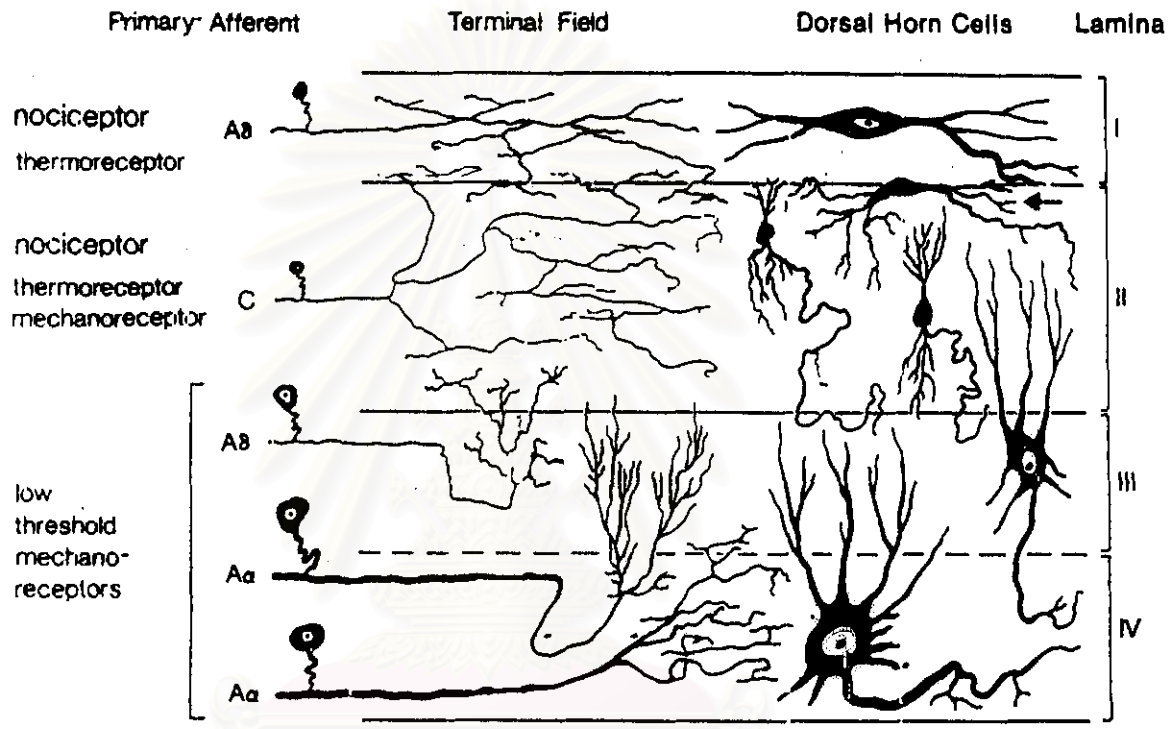

Figure 2. Summary of major components of the upper lamina of the spinal cord dorsal horn.

Physiological functions of serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is synthesized from the essential amino acids, tryptophan(Figure 3,4). The human body contains about 10 mg of 5-HT, 90% of which is in the enterochromaffin cells of the gastrointestinal tract, the remainder in the brain and platelets (Douglas, 1980; Stephen, 1994).

Central nervous system. A multitude of brain functions are influenced by 5-HT, including sleep, cognition, sensory perception, motor activity, temperature regulation, nociception, appetite, sexual behavior, and hormone secretion. The majorily of serotonergic neurons are located within the raphe nuclei in the medulla (Figure 5). In these sites, 5-HT serves as an inhibitory neurotransmitter (Messing and Lytle, 1997). An extensive body of evidence implicates bulbospinal serotonergic projection in pain modulation (Le Bars, 1988). The analgesic action of systemic opiates can be at least transiently reduced by depletion of 5-HT by inhibition its synthesis (Tenen, 1968), by neurotoxic destruction of spinal 5-HT terminal with 5,7-dihydroxytryptamine (Vogt, 1974) or by lesions of medullary regions which contain 5-HT neurons (Robert, 1988). The analgesia produced by intracerebral morphine can be partially blocked by intrathecal methysergide, a nonselective 5-HT antagonist (Yaksh, 1979). Iontophoresis of 5-HT inhibits the response of dorsal horn neurons to noxious stimuli (Rance and Yu, 1976; Headley et al., 1978; Jordan et al., 1979) and when 5-HT is applied directly to the spinal cord it produces analgesia (Hylden and Wilcox, 1983; Schmauss et al., 1983; Solomon and Gebhart, 1988).

Cardiovascular system. The classical response of blood vessels to 5-HT is constriction, particularly in the splanchnic, renal, pulmonary, and cerebral

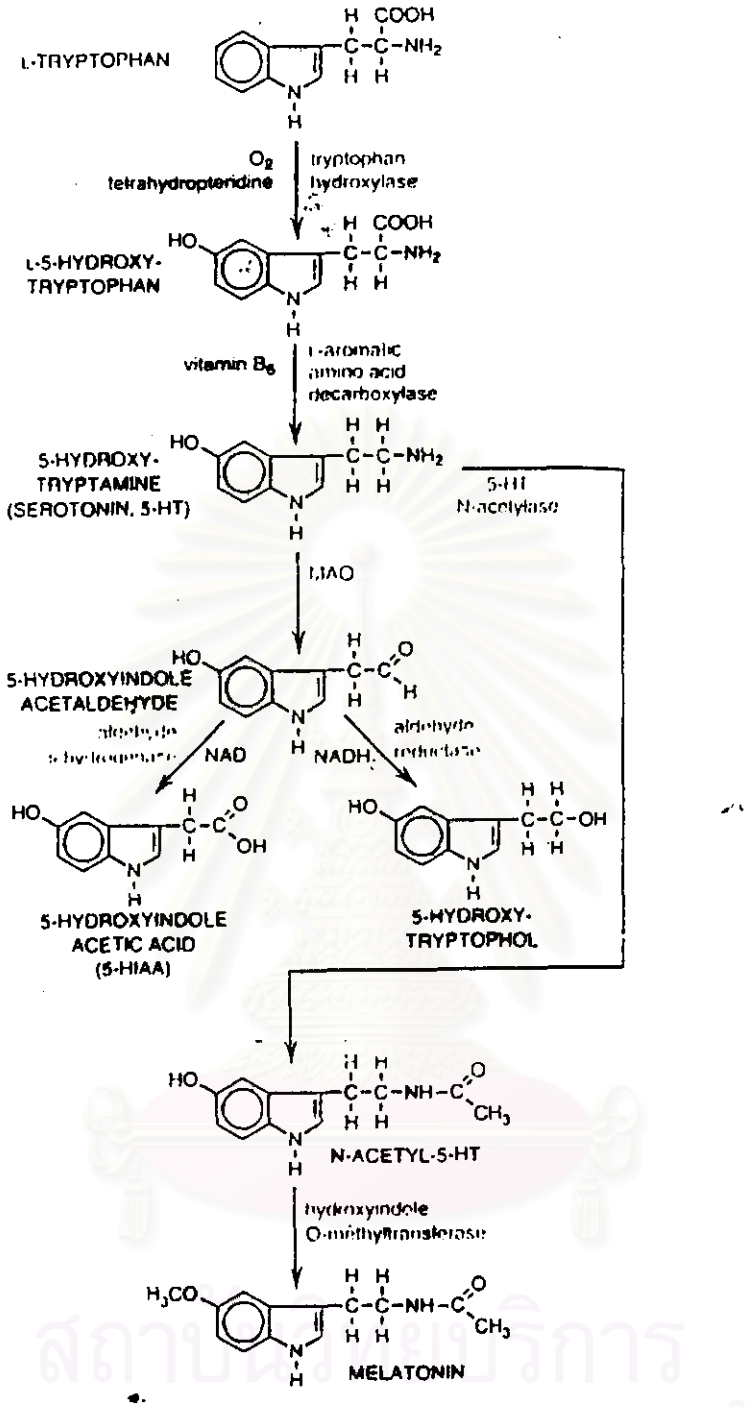

Figure 3. The biosynthesis and catabolism of serotonin.

vasculatures. Serotonin also induces a variety of cardiac responses depending receptor subtypes, these response involved stimulation of inhibition of autonomic activity, or dominance of reflex responses to 5-HT (Saxena and Villalon, 1990). Thus, 5-HT has positive inotropic and chronotropic actions on the heart that may be blunted by simultaneous stimulation of afferent nerves from baroreceptors and chemoreceptors. An effect on vagus nerve endings elicits the Bezold-Jarisch reflex, causing extreme bradycardia and hypotension. The local response of arterial blood vessels to 5-HT also may be inhibitory, the result of the release of endothelium-derived relaxing factor (EDRF) and prostaglandins and blockade of norepinephrine release from sympathetic nerves. On the other hand, 5-HT amplifies the local constrictor action of norepinephrine, angiotensin II, and histamine, which reinforce the hemostatic response to 5-HT (Gerson, 1991).

Enterochromaffin cells. Enterochromaffin cells, identified histologically, are located in the gastrointestinal mucosa, with the highest density found in the duodenum. These cells synthesize 5-HT from tryptophan and store 5-HT and other autacoids such as substance P and other kinins. Basal release of enteric 5-HT is augmented by mechanical stretching, such as that caused by food or the administration of hypertonic saline, and also by efferent vagal stimulation. 5-HT probably has an additional role in stimulating motility via the myenteric network of neurons, located between, the layers of smooth muscle (Gerson, 1991). The greatly enhanced secretion of 5-HT and other autaciod in malignant carcinoid leads to a multitude of cardiovascular, gastrointestinal, and CNS abnormalities.

Gastrointestinal tract. Enterochromaffin cells in the mucosa appear to be the location of the synthesis and most of the storage of 5-HT in the body

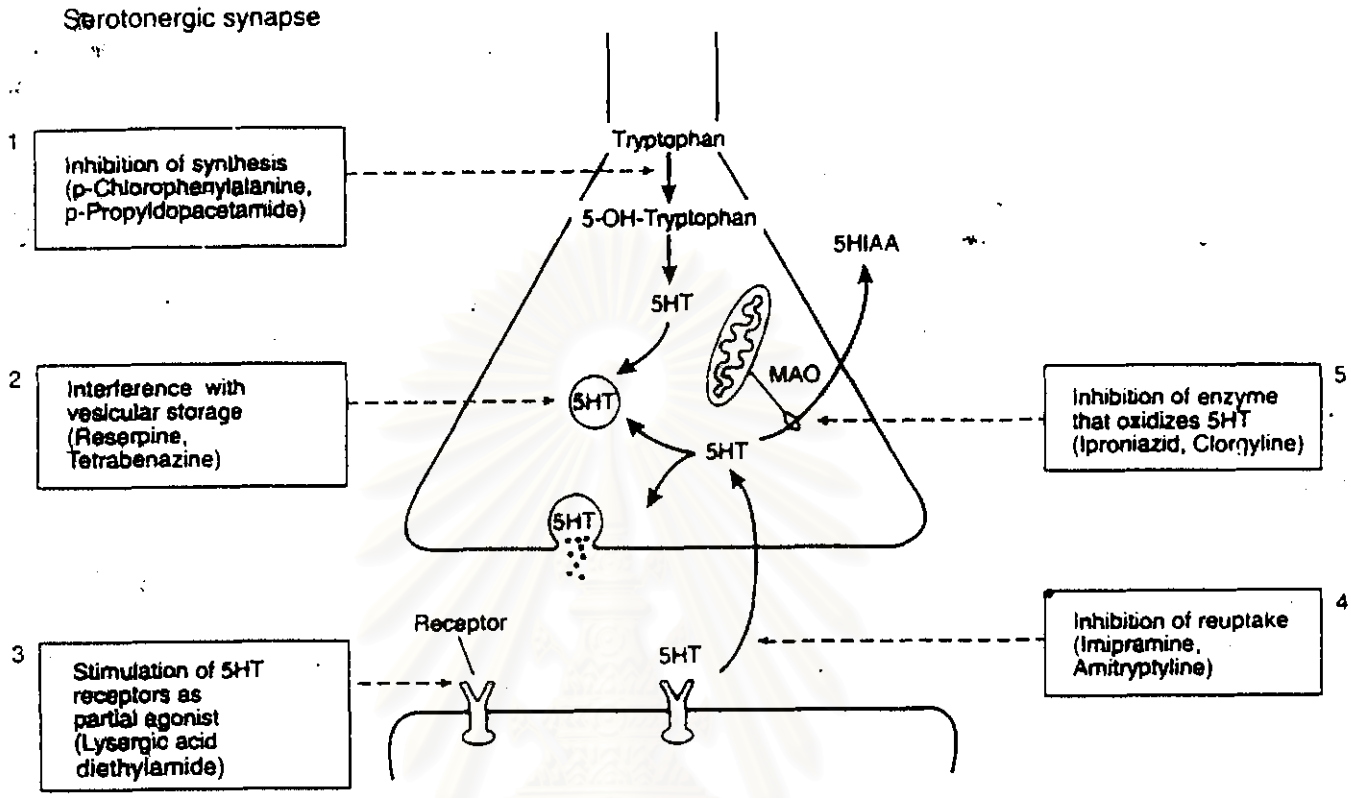

Figure 4. Key steps in serotonergic transmission

and are the source of circulating 5-HT. Serotonin released from these cells enters the portal vein and is subsequently metabolized by monoamine oxidase-A (MAO-A) in the liver (Gillis, 1985). 5-HT that survives oxidation in the liver is rapidly removed by the endothelium of lung capillaries and then inactivated by MAO. Serotonin released by mechanical or vagal stimulation also acts locally to regulate gastrointestinal function. Motility of gastric and intestinal smooth muscle may be either enhanced or inhibited (Dhasmana et al., 1993) via at least six subtypes of 5-HT receptor. The stimulatory response occurs at nerve endings on longitudinal and circular enteric muscle (5-HT_4 receptor), at postsynaptic cells of the enteric ganglia (5-HT_3 receptor), and by direct effects of 5-HT on the muscle cells (5-HT_{2A} receptor in intestine and 5-HT_{2B} receptor in stomach fundus). In esophagus, 5-HT acting at 5-HT_4 receptors causes either relaxation or contraction, depending on the species. Abundant 5-HT_3 receptors on vagal and other afferent neurons and on enterochromaffin cells play a pivotal role in emesis (Grunberg and Hesketh, 1993). Serotonergic terminals have been described in the myentric plexus. Release of enteric 5-HT occurs in response to acetylcholine, noradrenergic nerve stimulation, increases in intraluminal pressure, and lowered pH (Gershon, 1991), triggering peristaltic contraction.

Platelets. 5-HT is not synthesized in platelets but is taken up from the circulation and stored in secretory granules by active transport, similar to the uptake and storage of norepinephine by sympathetic nerve terminal. Thus, Na^+ -dependent transport across the surface membrane of platelets is followed by uptake into dense core granules via an electrochemical gradient generated by a H^+-translocating ATPase.

A gradient of 5-HT as high as 1000:1 with an internal concentration of 0.6 M in the dense core storage vesicles can be maintained by platelets.

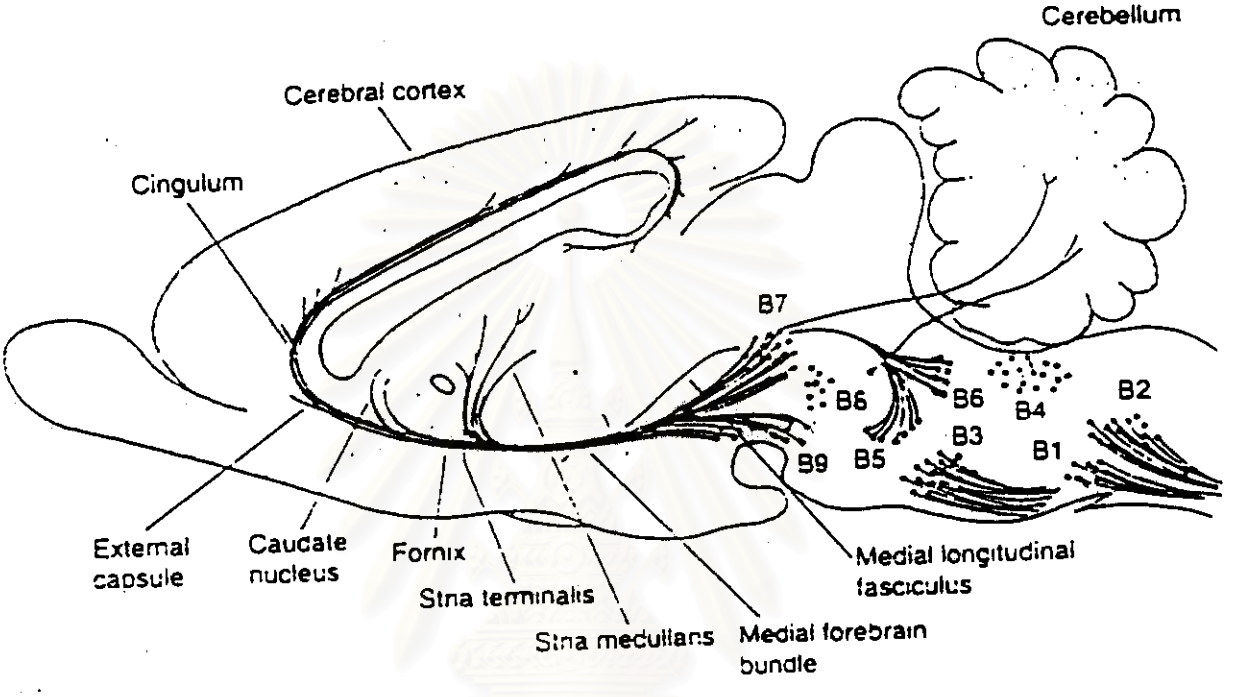

Figure 5. Schematic drawing depicting the location of the serotonergic cell body groups in a sagittal section of the rat central nervous system and their major projections.

Measuring the rate of Na^+ -dependent 5-HT uptake by platelets provides a sensitive assay for 5-HT uptake inhibitors. The main function of platelets is to plug holes in injured endothelial cells. Conversely, the functional integrity of the endothelium is critical for the action of platelets(Furchgott and Vanhoutte, 1989). The endothelial surface is exposed constantly to platelets, because the shear forces of circulating blood favor centrifugal stratification of platelets (Gibbons and Dzau, 1994). Release of EDRF (nitric oxide) antagonizes the vasoconstrictor action of thromboxane and 5-HT (Furgotte and Vanhoutte, 1989). The net effect of platelet aggregation is critically determined by the functional status of the endothelium (Hawiger, 1992; Ware and Heistad, 1993). When platelets make contact with injured endothelium, they release substances that promote platelet adhesion and release of 5-HT, including ADP, thrombin, and thromboxane A_2. Serotonin binding to platelet $5\text{-}HT_{2A}$ receptors elicits a weak aggregation response that is markedly enhanced in the presence of collagen. If the damaged blood vessel is injured to a depth where vascular smooth muscle is exposed, 5-HT exerts a direct constrictor effect, thereby promoting heamostasis. Locally released autacoids (thromboxane A_2 , kinin, and vasoactive peptides) enhance this action.

Table 1 Operational characteristics of 5-HT receptors

Receptor type	Subtypes	Location	Response	Agonist	Antagonist
5-HT_1	5-HT_{1A}	Neuronal, mainly in CNS	Neuronal hyperpolarization, hypotension	8-OH-DPAT,buspirone, 5-CT	WAY100135 (methiothepin)
	5-HT_{1B}	CNS and some peripheral nerves	Inhibition of neurotransMitter release	CP 93, 129, 5-CT	GR 127935 (metergoline, methiothepin (nonselective)
	5-HT_{1D}	Mainly CNS	Inhibition of adenylyl cyclase	Sumatriptan, L 694247, 5-CT	None (methiothepin)
	5-ht_{1E}	Only CNS	Inhibition of adenylyl cyclase	5-HT	None (methiothepin)
	5-ht_{1F}	Mainly CNS	Inhibition of adenylyl cyclase	5-HT	None (methiothepin)
5-HT_2	5-HT_{2A}	Vascular smooth muscle, platelets, lung, CNS, gastrointestinal tract	Vasoconstriction, platelete aggregation, bronchostriction	α-methyl-5-HT, DOI	Ketanserin, cinanserin, pirenperone

Receptor type	Subtypes	Location	Response	Agonist	Antagonist
	$5\text{-}HT_{2B}$	Mainly peripheral ?	Rat stomach fundic muscle contraction	α-methyl-5-HT, DOI	SB 200646 (also $5\text{-}HT_{2C}$ antagonist)
	$5\text{-}HT_{2C}$	CNS (high density in choriod plexus)	↑ phospho-inositide turnover	α-methyl-5-HT, DOI	Mesulergine (also $5\text{-}HT_{2A}$ antagonist)
$5\text{-}HT_3$		Peripheral and central neurons	Depolariza-tion	2-methyl-5-HT, *m*-chlorophenyl-biguanide	Ondansetron, tropisetron
$5\text{-}HT_4$		Gastrointes-tinal tract, CNS, heart, urinary bladder	Activation of Acetylcho-line release in gut, tachycardia, ↑cAMP in CNS neurons	Metoclopra-mide, renzapride (usually partial agonists) relative to 5-HT	GR 113808, SB 204070, tropisetron (weak)
$5\text{-}ht_{5A}$ and $5\text{-}ht_{5B}$		CNS	Not Known	5-HT	Methiothe-pin

(Modified from Hoyer et al., 1994)

General review of serotonin receptor types 1 and 2

In the relatively few years after the first unifying scheme for naming and classifying 5-HT receptors (Bradley et al; 1986), rapid and extensive advances in the development and application of gene cloning techniques have led to the discovery of many new 5-HT receptors. This has encouraged debate on the need to look beyond operational criteria as a primary basis for classification and prompted a number of proposals for a new taxonomy founded upon receptor structure (Frazer et al., 1990; Hartig, 1989; Peroutka, 1993) (Table 1). Obviously, the molecular properties of a receptor provide fundamental information for identification, but its recognize and transducer properties play an equally important part in defining its unique characteristics. This is abundantly clear in the case of the 5-HT_{1B} and 5-HT_{1D} (receptors, which are the products of equivalent genes in rodents and non-rodents respectively i.e. they fulfill the same physiological functions, yet they display highly species-specific pharmacological profiles (Oksenberg et al., 1992).

Serotonin Club Receptor Nomenclature Committee recently proposed a new nomenclature for 5-HT receptors which requires three fundamental properties of a receptor to be described to ensure a robust classification (Figure 7); its operational (drug related), transductional (receptor-coupling) and structural (primary amino acid sequence) (see Table 2)

5-HT_{1A} Receptors

1. Distribution and function. The hippocampus contains a high density of 5-HT_1 sites, most of which belong to the 5-HT_{1A} subtype. Other brain areas are enriched in 5-HT_{1A} sites, including the septum, some of the amygdaloid, and raphe nuclei, particularly the dorsal raphe (Marcinkiewicz et al.,1984; Radja et al., 1991). Many of these regions are components of the pathways involved in the modulation of emotion, the limbic system. This distribution is common to

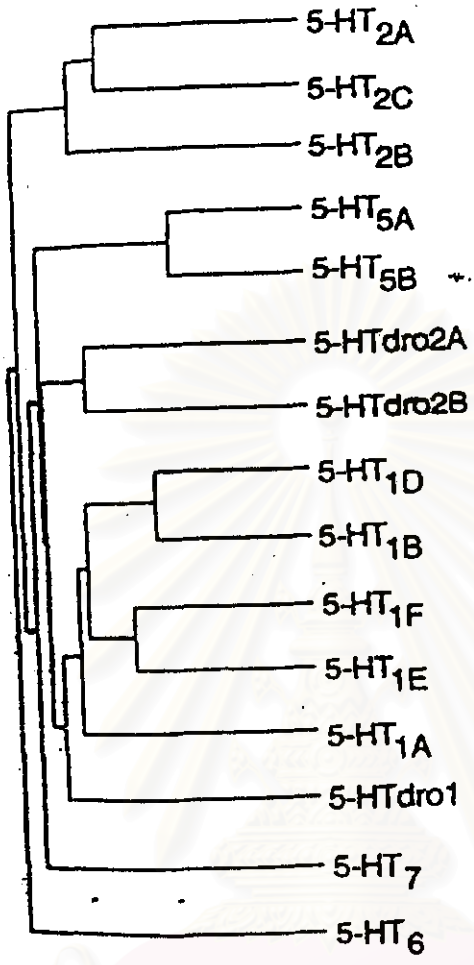

Figure 6. Dendogram analysis of 5-HT receptors of the G-protein-couple family.

several mammals, including humans (Hoyer et al., 1986; Pazos et al., 1987). The predominance of 5-HT_{1A} receptors in this system suggests that the reported effects of 5-HT and 5-HT receptors ligands in emotional could be mediated by 5-HT_{1A} receptors (Iverson, 1984), the presence of high densities of 5-HT_{1A} receptors in the raphe nuclei indicates that 5-HT can modulate the activity of serotonergic neurons. 5-HT_{1A} receptors are also present in the neocortex, the hypothalamus, and the substantia gelatinosa of the spinal cord. The localization of 5-HT_{1A} receptors in these areas suggests that 5-HT_{1A} mechanisms could also be involved in the functions in the hypothalamus, the regulation of proprioception and in integrative functions of the neocortex.

Activation of somatodendritic autoreceptors causes a reduction in 5-HT synthesis, release and electrical activity (De montigny and Blier, 1992). Destruction of serotonergic neuronal cell bodies by lesions have shown that the cell bodies carry 5-HT_{1A} receptors sites (Verge et al., 1986). Interestingly, no alterations in 5-HT_{1A} receptor-binding sites were seen after such lesions were created in forebrain areas, including the neocortex and hippocampus. A possible explanation is that the density of presynaptic 5-HT_{1A} receptors in forebrain region is low. Destruction of areas such as the hippocampus, using kainic acid, causes a selective degradation of pyramidal cells and interneurones, accompanied by the loss of in 5-HT_{1A} -binding sites (Hall et al ., 1985) This suggests that hippocampal 5-HT_{1A} receptors are essentially postsynaptic. The density of 5-HT_{1A} receptors in the hippocampus of patients with Alzheimer's disease is decreased in parallel with the loss of pyramidal cells. Thus, 5-HT_{1A} receptors in the hippocampus are probably postsynaptic to serotonergic afferents as suggested from the lesion experiments (Cross et al., 1984, 1988).

The activation of central 5-HT_{1A} receptors induced a behavioral syndrome, which is characterized by flatbody posture, reciprocal forepaw treading, and head weaving (Tricklebank, 1985). Typically, the administration

TABLE 2 Criteria for receptor characterization

Criteria	Definition
Operational	
a.Selective agonists	Agonist with unique or high selectivity for the receptor compared with their potencies at other receptors need to be identified. Their relative equieffective molar concentration ratios should be determined and a rank order of agonist potencies for the receptor established.
b. Selective antagonists	Receptor-blocking drugs (antagonists) are needed that can antagonize the action of agonists by blocking the receptor. It should be determined whether these antagonists are selective for the one type or subtype of receptor or not and what their respective equilibrium dissociation constants (affinity measures) are for their interaction at the receptor.
c.Ligand-binding affinities	Dissociation constants for ligands (selective agonists and antagonists) in binding studies should correlate with corresponding data from functional studies. Autoradiography also receptor distribution studies.
Structural d. molecular structure	The amino acid sequence of the receptor protein provides definitive evidence of receptor identity. However , receptors that are structurally different may not necessarily be different in operational terms and vice versa. Relative homologies of receptor proteins can also provide useful data for classification purposes , enabling definition of families and subfamily groups (Harting et al., 1992)
Transductional e.Intracellular transduction mechanisms	Important information that further defines the receptor super-family (i.e., ligand-gated ion channel or G-protein linked).It also involves definition of the nature of the G-protein linkage, if any , that may be indicative of the nature of the intracelluar protein structure of the receptor itself.

The scheme is modified from Coleman and Humphey, 1993, and Humphey et al., 1993.

of low dose of 8-OH-DPAT induces these behaviors which can be antagonized by compounds such as spiperone , BMY 7378, NAN 190, SDZ 216525, and (β-adrenoceptor antagonists such as pindolol, propranolol or alprenolol (Lucki, 1992). 5-HT_{1A} receptors agonists such as 8-OH-DPAT, gepirone, buspirone, ipsapirone also cause hyperphagia which can be effective antagonized with spiperone or pindolol. A variety of 5-HT_{1A} receptors agonists, especially those considered to be agonists, such as buspirone, gepirone, ipsapirone or tandospirone, have anxiolytic effects in animal models of anxiety (Traber and Glaser, 1987). Such compound are being, or have already been, developed as anxiolytic drugs, although clinical data with such drugs suggests an additional antidepressant activity; this is not unexpect because some of these compounds are active in animals models for depression, such as the forced swimming test (Cervo et al., 1988, Wieland and Lucki, 1990). Finally, variety of agonists such as 8-OH-DPAT, flesinoxan, urapidil, and 5-methyl-urapidil produce a decrease in blood pressure and heart rate by activation of central 5-HT_{1A} receptors (Doods et al., 1988; Dreteler et al., 1990, 1991).

2.Receptor and transduction. Lefkowitz's group, screening a human library with probes for the β_2 adrenoceptor, isolated the so-called clone G21 (Kobilka et al., 1987), which was subsequently shown to be the gene coding for the human 5-HT_{1A} receptor (Fargin et al., 1988). G21 is intronless, and the corresponding protein has a predicted 421 amino acids. The rat 5-HT_{1A} receptor gene has also been cloned (Albert et cal., 1990) and the receptor has 99% sequence homology with the human equivalent in the putative transmembrane regions (TMRs).

The first described 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated adenylyl cyclase in rat and guinea pig hippocampus (De Vivo and Maayani1985, 1986). Similar finding with forskolin-and vasointestinal polypeptide-stimulated adenylyl cyclase were reported in mouse and ginea pig

hippocampus cells or membranes (Weiss et al., 1986; Bockaert et al., 1987). In calf hippocampus, the rank order of potency of a large number of agonists and antagonists to inhibit forskolin-stimulated adenylyl cyclase correlated highly significantly with 5-HT_{1A} binding (Schoeffter and Hoyer, 1988). The G-protein coupling appears somewhat paradoxical, because in the hippocampus, 5-HT_{1A} receptors appear to mediate both stimulation and inhibition of adenylyl cyclase activity (Shenker et al., 1983, 1985, 1987). It would seem that either 5-HT_{1A} receptors are able to couple to at least two different G-proteins (G_S and Gi) in the same tissue (although not necessarily in the same cell) or, alternatively, stimulation and inhibition of adenylyl cyclase are mediate by two closely related but different receptors, which are difficult to distinguish pharmacologically. Hypothetically, the situation could be analogous to that with 5-HT_{2C} and 5-HT_{2A} receptors, which are very similar, both in terms of structure and pharmacology, and many the available ligands do not distinguish between the two receptors.

5-HT_{1B} Receptors

1. Distribution and function. The basal ganglia (Pazos and Palacios, 1985), especially the globus pallidus, and the pars reticulata of the substantia-nigra show high densities of 5-HT_{1B} sites. In the rat brain these sites are of the 5-HT_{1B} subtype, as assessed by their pharmacological profile (Pazos and palacios ,1985). In contrast to the situation in rats and mice, [^{3}H]5-HT binding in the basal ganglia of other mammals displays a pharmacological profile characteristic of 5-HT_{1D} sites. There is no evidence from binding studies for the presence of 5-HT_{1B} sites in guinea-pig, pig, calf, rabbit, dog, monkey, human, and pigeon brain. In autoradiographic studies, sumatriptan displaced [^{3}H]5-HT binding form 5-HT_{1B} sites in rat brain and 5-HT_{1D} sites in monkey and human brain (Waeber et al., 1989c). It has been shown, using the recently introduced 5-HT_{1B}/5-HT_{1D} ligand, [^{125}I]GTI, that the distribution of 5-HT_{1B} sites in rat brain similar to that of 5-HT_{1D} sites in guinea pig and human brain, with the

highest concentration in substantia nigra, globus pallidus, dorsal subiculum and superior colliculi (Segu et al., 1991; Boulenguez et al., 1992; Palacios et al., 1992). 5-HT_{1B} (5-$HT_{1D\beta}$) receptor mRNA has been reported in raphe nuclei, striatum, cerebellum, hippocampus (pyramidal cell layer of CA1), entorhinal and cingulate cortex (layer IV), subthalamic nucleus, and nucleus accumbens but not in the substantia nigra (Voigt et al., 1991; Maroteaux et al., 1992; Jin et al., 1992).

In the rat, lesion experiments have shown that 5-HT_{1B} receptors could be presynaptically localized on the terminals of the striatal intrinsic neuron that innervate the substantia nigra pars reticulata, because destruction of caudate neurons results in a dramatic decrease of binding in the substantia nigra pars reticulata (Hamon et al., 1990). The lesion of dopaminergic neurons in the substantia nigra pars compacta does not induce a decrease of 5-HT_{1B}-binding sites. It appears that 5-HT_{1B} receptors are localized on cells controlling the activity of the basal ganglia, but that they are not linked to the dopaminergic innervation. Presynaptic heteroreceptors may predominate, because lesions of serotoniergic neurones do not result in significant losses of 5-HT_{1B} binding in most areas examined.

2.Receptor and transduction. Voigt et al., (1991) and Adham et al., (1992) identified the 5-HT_{1B} receptor using a probe derived from the human 5-$HT_{1D\beta}$ receptor clone. The rat receptor gene is intronless, encoding for a 386-amino acid protein, and has 96% homology in the TMR with the equivalent human clone, but the rat receptor exhibits the typical 5-HT_{1B} operational profile. Similarly, a mouse 5-HT_{1B} receptor has been cloned (Maroteaux et al., 1992); these receptors (human 5-$HT_{1D\beta}$ and rat/mouse 5-HT_{1B}) represent species homologeus, as suggested earlier on the basis of their distribution in brain from a variety of species (Hoyer and Middlemiss, 1989).

5-HT_{1B} receptor have been shown to be negatively coupled to adenylyl cyclase in homogenates of rat substantia nigra, which predominantly possess a high density of 5-HT_{1B} sites (Bouhelal et al., 1988). In this preparation, the rank order of potency of both agonists and antagonists correlates well with affinity values for 5-HT_{1B}-binding sites (Bouhelal et al., 1988; Schoeffter and Hoyer, 1989a).Similar finding have been reported in a hamster lung cell line (Seuwen et al., 1988), in which the mitogenic effects of 5-HT could be related to inhibition of adenylyl cyclase activity. Cells transfected with rat and mouse 5-HT_{1B} receptor have been shown to be linked to inhibition of adenylyl cyclase activity and to display are operational profile typical of the 5-HT_{1B} receptor (Adham et al., 1992; Maroteaux et al., 1992).

5-HT_{1D} Receptors

1. Distribution and function. 5-HT_{1D} receptors have been to exist found to exist in the brain of a range of non-rodent mammalian species including guinea pig, rabbit, dog, pig and human (Heuring and peroutka, 1987; Waeber et al., 1988; Hoyer and Schoeffter, 1988; Herrick-Davies and Titeler, 1988; Beer et al., 1992; Maura et al., 1993). 5-HT_{1D} receptor reflects the distribution and function of the 5-HT_{1B} receptor found in the rodent. There is evidence that 5-HT_{1D} receptors do exist in the rat, although radioligand-binding studies would suggest that their concentration is very low (Herrick-Davies and Titeler, 1988; Bruinvels et al., 1993b).

The regional distribution of 5-HT_{1D} receptors in non-rodent species appears similar to that of the 5-HT_{1B} receptors in rodent, with the highest density in the substantia nigra, basal ganglia, and nigrostriatal pathway and a lower density in the hippocampus, raphe, and cortex (Waeber et al., 1990). However, it should be appreciated that radioligand-binding techniques do not currently allow the differentiation of $5\text{-HT}_{1D\alpha}$ and $5\text{-HT}_{1D\beta}$ receptors. [^{125}I]GTI

largely labels 5-$HT_{1D\beta}$ receptors in human cerebral cortex was reported by Beer and Middlemiss (1993).

The 5-HT_{1D} receptor was first identified as mediating inhibition of 5-HT release from cortical nerve terminals of the guinea pig brain (Middlemiss et al., 1988). It was shown that potencies of a variety of agonists and antagonists at the 5-HT_{1D} receptor mediating inhibition of adenylyl cyclase correlated very significantly with their effects on [^{3}H]5-HT release in pig cortex slices (Schlicker et al., 1989). Similar findings have been reported in guinea pig and rabbit brain (Limberger et al., 1991).

As is the case for 5-HT_{1B} receptors, 5-HT_{1D} receptors also appear to function as heteroreceptors, as judged by studies of nonserotonergic nerves where 5-HT appears to inhibit release of glutamate from guinea pig hippocampal synaptosomes (Raiteri et al., 1986; Harel-Dupas et al., 1991).

2.Receptor and transduction. Primers derived from the putative canine RDC4 receptor (377 amino acids) which has limited homology (55%) with human 5-HT_{1A} receptor (Libert et al.,1989) were used in the polymerase chain reaction to find the human equivalent that has 93% homology in the TMR. This human receptor exhibits typical 5-HT_{1D} receptor operational characteristics (Hamblin and Metcalf, 1991; Branchek et al ., 1991; Weinshank et al., 1992) and is a single protein of 377 amino acids. When transfected into mammalian cells, the RDC4 gene also exhibited a 5-HT_{1D}-type pharmacology (Maenhaut et al., 1991; Zgombick et al., 1991). A rat equivalent gene, encoding for a 374-amino acid protein (95% homology in the TMRs), has been cloned (Hamblin et al., 1992, Bach et al., 1993); this receptor has 5-HT_{1D} like characteristics, expect that drugs such as ritanserin and ketanserin are reported to have high affinity for this site. In addition, it was noted that the mouse equivalent have been cloned in RDC4. These clones (derived from RDC4) were named 5-HT_{1D} by Hartig et al.

(1992), and it is important is note that , so far , none of these cloned receptors exhibits a 5-HT_{1B} pharmacology. It would seem that the RDC4 related gene products are expressed at very low level (Libert et al., 1989; Weinshank et al., 1992) or at least that the mRNA levels are very low (Jin et al., 1992; Bruinvels et al., 1993a, b).

In humans, a second receptor relatively similar to terms of sequence (77% in the TMR) has been cloned. The human gene product which has 390 predicted amino acids (named 5-$HT_{1D\beta}$) is the species homologue of the rodent a 5-HT_{1B} receptor (97% homology). However, the 5-HT_{1D} receptor exhibits a 5-HT_{1D} pharmacology that is distinguishable from that of the 5-$HT_{1D\alpha}$ clone with ligands used to date (Weinshank et al., 1992; Levy et al., 1992b; Jin et al., 1992), The obvious question is which of the two receptors (5-$HT_{1D\alpha}$ or 5-HT_{1D} β is relevant to the various pharmacological effects of 5-HT_{1D} receptors activation described extensively in the literature. The parallel between 5-HT_{1B} and 5-HT_{1D} receptors (similarly to functions and distributions across species) and the mild lower density of 5-$HT_{1D\alpha}$ receptor mRNA and protein would suggest that the 5-$HT_{1D\beta}$ receptor is the counterpart of what has been described in functional and chemical studies as 5-HT_{1D} receptors. Evidence has been presented that this may indeed be so in human artery because the operational characteristics of arterial 5-HT_1 receptors are closer to those of 5-$HT_{1D\beta}$ than those of 5-$HT_{1D\alpha}$ receptors (Hamel et al., 1993a,b; Kaumann et al., 1993). This may also be the case for other 5-HT_{1D} models (e.g., inhibition of cAMP production in calf substantia nigra, inhibition of 5-HT release in non-rodent or porcine coronary artery contraction) where compounds such as ketanserine (or ritanserin) when tested are devoid of activity (it seems that these two antagonists have significant affinity for 5-$HT_{1D\alpha}$ receptors).

Activation of 5-HT_{1D} receptors lead to inhibition of forskolin-stimulated adenylyl cyclase activity in calf of guinea pig substantia nigra (Hoyer and

schoeffter,1988; Schoeffter et al.,1988; Waeber et al., 1989d), which contain a high proportion of 5-HT_{1D} sites(Waeber et al.,1988c, 1989a). Most studies performed with cells transfected with 5-$HT_{1D\alpha}$ receptors (both 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (types) show that these receptors are indeed negatively coupled to adenylyl cyclase. However, the canine RDC4 clone, depending on the type of cell system used, can be linked positively (Maenhaut et al., 1991) or negatively to adenylyl cyclase (Zgombick et al.,1991). It appears that depending on the cell line used for the expression of 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors, promiscuous coupling can be observed, e.g., inhibition of adenylyl cyclase and stimulation of calcium mobilization (Zgombick et al., 1993).

5-HT_{2A} Receptors

1. Distribution and function. 5-HT_{2A} receptors are widely distributed in peripheral tissue (Bradly et al., 1986). The effects mediated by these receptors include contractile responses in many vascular smooth muscle preparations (e.g., rabbit aorta, rat caudal artery, dog retrosplenic vein), contractile response in bronchial, uterine, and urinary smooth muscle, and part of the contractile effect of 5-HT in guinea pig ileum. In addition, platelet aggregation and increased capillary permeability can be included as 5-HT_{2A} receptor-mediated action. The only central actions classified by Bradley and colleagues as 5-HT_{2A} receptor mediated were some behavioral effects in rodents (head twitch, wet-dog shake) and neuronal depolarization (rat facial motorneuron , rat spinal motorneurons, cat preganglionic sympathetic neurons).

Nevertheless, 5-HT_{2A} receptors are enriched in many areas of the cortex (Pazos et al., 1985, 1987b; Hoyer et al., 1986a). 5-HT_{2A} sites are also found in the claustrum, a region that is connected to the visual cortex, some components of the limbic system, particularly the olfactory nuclei, and parts of the basal ganglia. Attempts to determine the location of the cells expressing 5-HT_{2A} receptors in the neocortex, using lesion experiments, have suggested that these

receptors are localized on the processes of intrinsic cells, because deafferentation and many other types of lesions do not result in changes in 5-HT_{2A} receptors densities (Leysen et al., 1983). On the other hand, cortical [^{3}H] Ketanserin-binding sites have been reported to be decreased in senile dementia of the Alzheimer type, paralleling the loss of somatostatin immunoreactivity (Cross et al., 1984, 1988). This suggests that 5-HT_{2A} receptors could be located on intrinsic somatostatin-containing neurons in the cortex.

In term of functions now characterized as being mediated by 5-HT_{2A} receptors, there has been considerable expansion, almost all being related to the CNS and neuroendrocrine actions. 5-HT_{2A} receptors mediate neuroexcitation in guinea pig cortical pyramidal neurons (Davies et al., 1987), rat raphe cell bodies (Roberts and Davies, 1989), and rat nucleus accubens neurons (North and Uchimura,1989).

2.Receptor and transduction. Pritchett et al.(1988) isolated the first cloned cDNA sequence encoding the complete 5-HT_{2A} receptor from a rat brain cDNA library. The similarities between 5-HT_{2A} and 5-HT_{2C} receptors in terms of second messengers and pharmacology guided the cloning strategy adopted by Pritchett et al.(1988) two oligonucleotides directed against two separarate amino acid residue sequences in the cloned 5-HT_{2C} receptor gene that had been characterized by Julius et al.(1988) were used to probe the rat brain cDNA library. The predicted 5-HT_{2A} receptor polypeptide contains seven TMRs and the amino acid sequence within the transmembrane regions is 80% identical with that of the 5-HT_{2A} receptor. The cDNA was transiently expression in a mammalian cell line; binding studies in membrane preparations from these cells confirmed the identify of the expressed 5-HT_{2A} receptors.

It is well established that 5-HT_{2A} receptors are linked to phosphatidylinositol turnover. This has been demonstrated in rat cortex, aortic

smooth muscle, and human platelets (Conn and Sanders-Bush, 1984, 1985; Rock et al., 1984; De Chaffoy et al., 1985; Doyle et al., 1986). The receptors are coupled to phospholipase C, and inositolphospholipid hydrolysis and Ca^{2+} mobilization are involved in the postreceptor events. It follows that the measurement of phosphatidylinositol turnover (e.g.accumulation of inositol 1-phosphate) can serve as a useful mean of monitoring functional effects of 5-HT_{2A} receptor activation in a variety of locations.

5-HT_{2B} Receptors

1. Distribution and function. The rat stomach fundic strip has been known for a long time to be exquisitely sensitive to 5-HT (Vane, 1959). However, this receptor whose activation leads to fundic smooth muscle contraction, has not been easy to characterize pharmacologically. It was originally classified as "5-HT_1-like". Although the fundus receptor shared some characteristics with the classical 5-HT_2 receptor, it was clear that it was not a 5-HT_{2A} receptor (Clineschmidt et al.,1985; Cohen and Wittenauer, 1987). Based on the rank order of potency of a variety of agonists, the fundus receptor was shown bear resemblance to the 5-HT_{2C} receptor (formerly 5-HT_{1C}) (Buchheit et al., 1986); however more through investigations showed that the fundus receptor was not a 5-HT_{2C} receptor (Cohen, 1989; Kalkman and Fozard, 1991a). Furthermore, it could be demonstrated that 5-HT_{2C} mRNA is not to be found in rat fundus preparations (Baez et al., 1990; Foguet et al., 1992b). Eventually, the situation was clarified by the cloning of the rat and mouse "fundic" receptor (Foguet et al.,1992a,b; Kursar et al., 1992). Kursar et al.(1992) named this receptor 5-HT_{2F} (fundus). For reasons delineated above, it have recommended naming the fundus receptor 5-HT_{2B} ,because the classical 5-HT_2 receptor and the 5-HT_{1C} receptors are now termed 5-HT_{2A}and5-HT_{2C} respectively (Humphey et al., 1993)Little is known about the distribution of the receptor in rat. For instance, Northern blot analysis did not reveal the presence of the 5-HT_{2B} receptor in the rat brain (Kursar et al., 1992; Foguet et al. 1992) by a

quantitative polymerase chain reaction procedure were able to detect 5-HT_{2B} mRNA in a variety of tissues, including the fundus, gut, heart, kidney, and lung, and the some extent in brain. Loric et al., (1992) also cloned the mouse homologue of the rat receptor which appears to be expressed in mouse intestine and heart and to a lower extent in brain and kidney.

2.Receptor and transduction. Foguet et al. (1992b) screened a mouse genomic library and described a partial sequence of a G-protein-coupled receptor that was very similar to both 5-HT_{1C} and 5-HT_2 receptors (62 and 65%) with the same intron-exon boundaries. This receptor was called SRL and has a predicted size of 460 amino acids. By quantitative polymerase chain reaction, Foguet et al., (1992a) were then able to detect its presence in rat fundus mRNA and to clone the corresponding cDNA from a rat fundus cDNA library, Both groups reported a predicted amino acid sequence of 479 amino acids for the rat receptor, whereas Loric et al (1992) reported that there are 504 amino acids in the mouse receptor. Expression of SRL in *Xenopus* oocytes leads to activation of chloride channels, an effect that is probably mediated by the activation of phospholipase C (Foguet et al.1992a). Indeed, it has been shown that the recombinant fundus receptor is able to promote stimulation of phospholipase C activity (Kursar et al., 1992; Wainscott et al., 1993), but this has yet to be shown for the endogenous receptor (Cohen and Wittenauer, 1987; Secrest et al., 1993). Wang and colleagues (1993) found that the fundus 5-HT receptor appears to couple to a pertussis toxin-sensitive $G\alpha_Z$-like protein and in this regard may differ from other 5-HT_2 "receptor subtypes." Further studies with the natively expressed 5-HT_{2B} receptor are clearly desirable to determine whether or not this represents another example of cell-specific receptor coupling or a real difference in the transducer characteristics of members of the 5–HT_2 receptor group.

The human 5-HT_{2B} receptor gene has also been cloned (Ullmer et al., 1996). The human receptor protein is 80% homologous to the rat receptor, and

the intron /exon distribution in the gene is conserved in both species. The human receptor also couples to phospholipase C.

5-HT_{2C} Receptors

1.Distribution and function. The presence of high densities of binding sites in the choroid plexus was observed in early autoradiographic studies performed with 5-HT_1 receptor ligands such as [^{3}H]LSD and [^{3}H]5-HT but not 5-HT_2 receptor ligands except [^{3}H] mesulergine(Meibach et al., 1980; Pazos et al., 1984). Thus, it was assumed that these receptors belonged to the 5-HT_1class and were named 5-HT_{1C} (Pazos et al., 1984). These sites have been visualized in the choroid plexus of all of the mammalian species investigated thus far. The properties of these receptors are very similar regardless of the species studied. 5-HT_{2C} sites are enriched on the epithelial cells of the choroid plexus (Yagaloff and Hartig, 1985). Serotonergic nerve terminals are represent on the walls of the cerebral ventricles, and it has been suggested that 5-HT_{2C} receptors could regulate the composition and volume of the cerebrospinal fluid (Pazos et al., 1984). 5-HT_{2C} receptors are also present, although at lower densities than in the choroid plexus, in the limbic system and regions associated with motor behavior (Pazos and Palacios, 1985). Interestingly, 5-HT_{2C} sites appear to be more abundant in the basal ganglia of humans, particularly the globus pallidus and in the substantia nigra (Pazos et al., 1987a). Using in situ hybridization, Julius et al., (1988) observed high densities of 5-HT_{2C} receptor mRNA in the rat choroid plexus. 5-HT_{2C} transcripts were also found at significant densities in the olfactory nucleus, cingulate cortex, lateral habenula, and subthalamic nucleus (Mengod et al.,1990a).

2. Receptor structure and transduction. Lubbert and colleagues (1987a,b) identified the receptor gene expression cloning, and the sequence of the receptor of the rat was first described by Julius et al. (1988) contrast to 5-HT_1 receptors, but similarly to 5-HT_{2A} receptor and 5-HT_{2B} receptors, the gene

for the 5-HT_{2C} receptor predicted protein product of 460 amino acids) has introns and it is possible that different gene products can occur due to alternate splicing. The mouse and human homologues have been cloned and show 98% homology the TMRs (Yu et al., 1991; Saltzmann et al., 1991; Hoffman and Mezey, 1989). 5-HT_{2C} receptor activation in rat, mouse, and pig choroid plexus leads to the stimulation of phospholipase C activity and accumulation of inositol phosphates (Conn et al., 1986; Conn and Sanders-Bush, 1986; Hoyer, 1988b; Hoyer et al., 1989b).Interestingly, these studies confirmed what was indicated by radioligand-binding studies, i.e., a variety of so-called 5-HT_{2A} ligands acted as potent agonists of antagonists at 5-HT_{2C} receptors (Sahin-Erdemli et al., 1991b). In developing rat hippocampus, 5-HT_{2C} receptors mediate stimulation of phospholipase C activity (Claustre et al., 1990). In oocytes injected with 5-HT_{2C} receptor mRNA, 5-HT activates a Cl^- channel (Lubbert et al., 1987b). In general, cells transfected with 5-HT_{2C} receptors have consistently been reported to show activation of phospholipase C activity in response to 5-HT (Julius et al., 1988).

Reviews of 5-HT receptor subtypes and nociception

5-HT_1 receptors

A number of studies indicate that activation of 5-HT_1 receptors reduces nociceptive responsiveness in behavioral tests using a thermal noxious stimulus (Table 3). In the tail-flick and hot-plate tests, nociceptive sensitivity is reduced by 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), mCPP, 5-methoxy-3 (1,2,3,6-tetrahydropyridin-4-yl)-1H-indole (RU 24969) and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (Archer et al., 1987; Berge, 1982; Eide et al., 1988; Eide and Hole, 1989; Eide et al., 1990; Sawynok and Reid, 1994; Zemlan et al., 1980). 8-OH-DPAT is a selective 5-HT_{1A} agonist (Middlemiss and Fozard, 1983) and Ru 24969 is a combined 5-$HT_{1A,1B}$ agonist (Hoyer et al., 1990). Although mCPP has been considered a 5-HT_{1B} agonist, this compound also has affinity for 5-HT_{1A} (Schoeffter and Hoyer, 1989). 5-MeODMT is a non-selective agonist with affinity for 5-HT_{1A} and 5-HT_{1B} receptors, but weak affinity for 5-HT_2 receptors (Trickelband et al., 1985). In the tail-flick test 8-OH-DPAT reduces nociceptive responsiveness when given intrathecally (i.th.) (Eide et al., 1990; Eide and Hole, 1991) although not when given systemically (Eide et al., 1988; Fasmer et al., 1986). Another 5-HT_{1A} agonist, buspirone, reduced responsiveness in the rat hot-plate test (Bragin et al., 1989), and a 5-HT_{1B} agonist showed antinociceptive activity in the monkey (McKearney, 1989). In contrast to these reports, a single study reported that responsiveness was increased in the rat tail-flick test after i.th. injection of 8-OH-DPAT or RU 24969 (Ali et al., 1994; Solomon and Gebhart, 1988). A methodological problem with tests using thermal noxious stimuli (e.g. the tail-flick and hot-plate tests) is that changes in the skin temperature affect the behavioral responses recorded (Hole et al., 1990). It has been found in the tail-flick test that the tail-flick latencies depend on the skin temperature of the tail, and effects of 5-HT

agonists in this test may be due to changes in the skin temperature (Eide and Tjϕlsen, 1988).

The role of 5-HT_{1A} receptors in the control of nociception may depend on the type of noxious stimulation. Nociceptive responsiveness in the formalin test utilizing chemical noxious stimulation was increased by a low dose of 8-OH-DPAT (0.06 mg/kg) but reduced by a higher dose (1.0 mg/kg) (Fasmer et al., 1986). Others examining receptive field areas of withdrawal reflexes evoked by mechanical noxious stimulation found that the receptive field areas were increased by the agonists 8-OH-DPAT and 5-MeODMT (Murphy and Zemlan, 1990; Zemlan et al., 1983) and reduced by mCPP (Murphy and Zemlan, 1990). It is not possible, however, to exclude peripheral effects of the 5-HT agonists, because all the agonists were given systemically.

Substance P (SP) is a putative transmitter of primary afferent nociceptive neurons (Pernow, 1983) and induces a behavioral response indicative of noxious stimulation when injected i.th. The behavioral response to i.th. SP was reduced by i.th. administration of 8-OH-DPAT, mCPP or RU 24969.

The 5-HT_1 receptors in the spinal cord are particularly dense in the superficial layers of the dorsal horn (Hammon et al., 1990). Electrophysiological studies consistently indicate that stimulation of the 5-HT_1 receptors inhibits the transmission of nociceptive information at the spinal level. The 5-$HT_{1A,1B}$ agonist, RU 24969 applied iontophoretically inhibited the responses of dorsal horn neurons to noxious stimulation 5-HT_{1A} agonist 8-OH-DPAT induced a non-selective inhibition of neuronal response to noxious and non-noxious stimuli (El-Yassir et al., 1988). The responses of dorsal horn neurons to nociceptive stimulation were inhibited by brainstem stimulation probably by 5-HT_1 receptor activation since the inhibition was antagonized by a 5-HT_1 receptor blocker but not by a 5-HT_2 receptor blocker (El-Yassir and

Fleetwood-Walker, 1990). This receptor may involve in analysis elicited by brainstem stimulation. The dorsal horn nociceptive neurons that were inhibited by ventral medullary stimulation and by iontophoretically applied 5-HT were also inhibited by the 5-HT1A,1B,1C receptor agonist mCPP (Harris et al., 1986). Others have reported that the glutamic acid-induced firing of dorsal horn nociceptive neurons was inhibited by iontophoretic mCPP, but was unaffected by 8-OH-DPAT (Zemlan et al., 1988).

5-HT_2 receptors

Several lines of evidence suggest that stimulation of 5-HT_2 receptors may increase the transmission of nociception at the spinal level (Table 3), an effect that may be related to release of SP from presynaptic terminals. In preparations from the rat spinal cord the release of SP evoked by K^+ was increased by 5-HT probably by 5-HT_2 receptor activation (Iverfeldt et al., 1986). Intrathecally administerd 5-HT and the 5-HT_2 receptor agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and alpha-methyl-5-HT (alpha-Me-5HT) induced a similar response as i.th. SP, mimicking noxious stimulation (Eide and Hole, 1989; Eide and Hole, 1991; Wilcox and Alhaider, 1990), whereas i.th. 5-HT_1 or 5-HT_3 agonists failed to induce the behavioral response to i.th. 5-HT was reduced by a 5-HT_2 receptor blocker (Wilcox and Alhaider, 1990). DOI has high and selective affinity for 5-HT_2 sites and lower affinity at 5-HT_{1C} sites (Titeler et al., 1988). The behavioral response to i.th. 5-HT and DOI was blocked by SP receptor blockers, supporting the hypothesis that the response to 5-HT_2 agonists results from release of SP (Eide and Hole, 1991a; Eide and Hole, 1991b). On the other hand, the behavior response to i.th. SP that was inhibited by 5-HT_1 agonists was unaffected by DOI. Some data have reported that the electrophysiological responses of dorsal horn neurons to noxious stimulation are not affected by an iontophoretically applied 5-HT_2 receptor agonist but inhibited by 5-HT_1 agonists (El-Yassir et al.,1988). In this context, it is interesting that

several electrophysiological studies provide evidence that 5-HT_2 receptors mediate the excitatory effect of 5-HT and that 5-HT_{1A} receptors mediate the inhibitory effect of 5-HT (Aghajanian et al., 1990; Brandao et al., 1991). It has been found in man that the excitatory effect of 5-HT on peripheral afferent C-fibers supplying the ankle joint is probably mediated by 5-HT_2 receptors (Grubb et al., 1988). Some behavioral data also suggest that activation of 5-HT_2 receptors at the supraspinal level enhances nociceptive responsiveness in rats (Barder et al., 1989) and mice (Alhaider, 1991).

Activation of 5-HT_2 receptors may reduce the transmission of nociceptive information. The 5-HT_{2A} agonist DOI increased latencies in the tail-flick test in pentobarbital anaesthetized mice (Banks et al., 1988) and in awake mice (Eide and Hole, 1991a) and rats (Solomon and Gebhart, 1988). It is not known, however, whether the antinociceptive effect of i.th. DOI is due to an action on receptors other than the 5-HT_2 receptor subtype.

Table 3. Behavioral changes in nociceptive responsiveness depending on the 5-HT receptor subtypes activated.

Receptor Subtype	Receptor agonists	Type of noxious stimulation	Nociceptive responsiveness	References
5-HT_{1A}	8-OH-DPAT 5-MeODMT RU 24969 mCPP	Thermal	Reduced	Archer 1987, Berge1982 Bragin 1989 Eide 1988,1989, 1990, Fasmer 1986, Lin 1996, Sawynok 1994, Zemlan 1980
			Increased	Solomon 1988, Ali 1994
		Mechanical	Reduced	Danzebrink, 1991 Murphy 1990
			Increased	Murphy 1990, Zemlan 1983
		Chemical	Reduced	Eide 1992 Wilcox 1990
			Increased	Fasmer 1986
5-HT_{2}	alpha-Me-5-HT DOI	Thermal	Reduced	Banks 1988, Eide 1991, Solomon 1988
		Mechanical	Reduced	Danzebrink 1991
		Chemical	Increased	Eide 1991, Wilcox 1990

Gene induction following noxious stimulation

Immediate early genes (IEGs) were originally described as a class of genes rapidly and transiently in cells stimulated with growth factors without the requirement for de novo protein synthesis (Cochran et al., 1983); c-*fos*, c-*jun*, and other IEGs have been shown to be transcription factors and are differentially expressed in the central nervous system following specific types of stimulation (Wisden et al., 1990; Cole et al., 1989; Morgan 1991). Accumulating evidence suggests that IEG changes in gene expression with the nervous system signal long-term adaptation within particular neural pathways. c-*jun* and c-*fos* are members of a family of closely related proteins that posses a leucine zipper through which they form homo- or heterodimers that will bind to DNA and act as transcriptional regulators (Lamb and McKnight, 1991). However, the control of gene expression and protein activity is complete and may involve transcriptional, combinatorial, temporal, and posttranslational mechanisms, which can vary between different cell types (Karin and Smeal,1992; Jackson, 1992).

The c-*jun* and other *jun* proteins heterodimerize with c-*fos* or other *fos*-related proteins to form the AP1 transcription factor; with members of the cyclic adenosine monophosphate (AMP) response element binding protein/activating transcription factor (CREB/ATF) family of binding proteins. In this form, molecules such as c-*jun* and c-*fos* are able to bind to DNA domains in a sequence-specific manner and modulate gene transcription (Diamond et al., 1990; Vogt and Bos,1990). Different combinations of proteins can possess both an increased DNA binding activity and transactivating activity when compared with *jun-jun* homodimers. Following stimulation of neurons, neuronal cell lines, or glial cells with phorbol esters, polypeptide hormones, growth factors cytokines, or neurotransmitters, the expression of c-*jun* and

c-*fos* mRNA may follow IEG kinetics (Wisden et al., 1990; Sheng and Greenberg, 1990). This is characterized by a rapid but transient expression of mRNA following stimulation and is not dependent upon new protein synthesis. However, in certain situations the induction of c-*jun* expression can be both delayed and prolonged, as for example during the differentiation of F9 carcinoma cell with retinoic acid or in the neuronal response to axonal damage described below.(Yang-Yen et al., 1990; Jenkins and Hunt; 1991; Leah et al., 1991; Sleigh, 1992).

IEG expression *in vivo* appears to be tied to the physiologic context of the stimulus. The appearance of IEG product *in vivo* does not simply reflect a pattern of evoked activity but a pattern that is crucially related to the type of stimulation and the physiologic state of the animal. In other words, IEGs cannot be regarded simply as activity markers in vivo but perhaps as indicating the occurrence of a significant environment stimulation that requires a long term change in certain aspects of neuronal physiology. It was able to demonstrated that c-*fos* is expressed postsynaptically in dorsal horn neurons of the spinal cord following noxious stimulation (Hunt et al., 1987; Williams et al.,1989; Williams et al., 1990). The protein product appears within 1 to 2 hours poststimulation and c-*fos* positive neurons are restricted to laminae I and II (the substantia gelatinosa) of the dorsal horn, with some labeling in lamina V. It found that the type of stimulation was crucial for a change in gene expression within postsynaptic neurons of the dorsal horn.

Brief stimulation (5 sec) of high-threshold C or Aδ sensory fibers, but not low threshold A fibers, results in substantail long-term changes in spinal cord physiology. A similar brief noxious stimulus (chemical, heat, or mechanical) results, within 1 hour, in the induction in dorsal horn neurons of c-*fos* protein and in the mRNA and protein for a large number of other IEGs including members of the *jun* family (c-*jun*, *jun* B, and *jun* D), c-*fos*-related genes and c-

fos and the zinc finger-containing gene product NGFI-A (zif 268) (Hunt et al., 1987). The kinetics of induction can vary between the different genes (Herdegen et al., 1981; Fitzgerald, 1990) and be differentially modified by morphine pretreatment (Tolle et al., 1990). Induction of IEGs was never seen within sensory neurons in the dorsal root ganglion even following direct electrical stimulation. Similarly it have rarely seen c-*fos* immunoreactivity within neurons in areas of termination of large-diameter (low-threshold) primary sensory fibers, including the dorsal column nuclei and the ventral horn. The pattern of c-*fos* positive cells was established at birth and the number of labeled neurons was proportion to stimulus duration and intensity. These observations highlight the specificity of the gene induction within the spinal cord and suggest that it only occurs following stimulation that could be interpreted as injurious and was solely mediated by polymodal nociceptive C fibers and perhaps Aδ fibers.

However, longer poststimulus survival times revealed more complex pattern of molecular changes. Twenty-four hours after noxious heat stimulation or section of the peripheral nerve, the pattern of apparently monosynaptically induced c-*fos* in superficial neurons of the spinal cord gave way to a "second wave" of labeled cells now restricted to deeper laminae and distributed bilaterally within the cord (Williams et al., 1990). This second wave of c-*fos* activity was found to be independent of any further impulses from the primary afferent fibers, as it was not prevented by local anesthetic blockade of the injured sciatic nerve. This suggests that continuing primary afferent activity is not required for ongoing c-*fos* activation by neurons in the spinal cord. Instead the system becomes self sustaining (Williams et al.,1989; Williams et al., 1990). Thus, following a single noxious stimulation, a series of long-term molecular changes evolve over time and involve neurons several synapses removed from the initial monosynaptic excitation.

C-*fos* expression : a molecular marker of neuronal activity

The proto-oncogene : c-*fos* belongs to a large family of "immediate early onset" genes, activated in various cell types within minutes of stimulation by growth factors or neurotransmitter substances (Greenberg, 1985). These gene might play a role in the establishment of long-term functional change in the nervous system (Berridge, 1986; Goelet et al., 1986). Noxious stimuli increase the number of cell expressing c-*fos* protein within Rexed' s lamina I and II where unmyelinate c-*fos* terminate and spinothalamic projecting neurons predominate (Bullit, 1990; Gogas et al., 1991).

Interest in c-*fos* and pain was first provoked by the important findings in the rat spinal cord the proto-oncogene c-*fos* is rapidly expressed in the appropriated post-synaptic dorsal horn neurons for 24 hours following noxious heating or chemical stimulation of the periphery. Fos immunoreactivity in this model appear to the restricted to cells with neuronal morphology under light and electron microscopy (Hunt, 1987), and some can be retrogradely labelled from projection areas such as the thalamus and brain stem (Menetrey et al., 1989).

C-*fos* is expressed within neurons following voltage-gated Ca^{2+} entry into the cells (Morgan and Curran, 1986). Neuronal excitation leads to a rapid and transient induction of c-*fos* (Morgan et al., 1987). The protein product can be detected within neurons by immunohistochemical technique 20-90 minutes after neuronal excitation and disappear 4-6 hours later (Morgan et al., 1987). Once expressed, c-*fos* protein enters the cell nucleus and participates in protein complex that interacts with DNA (Sambucetti and Curran, 1986). Electron microscopy has known that neuronal excitation induces c-*fos* like immunoreactivity within the nuclei of neurons, but not within glia, ependymal or endothelial cells (Magnaini et al., 1989).

Recent advances in the understanding of the pathophysiology of migraine have included more complete descriptions of the pathways that are involved in head pain. C-*fos* expression within the trigeminal nucleus caudalis after instilling autologous blood into the subarachnoid space of rats has been studied (Nozaki et al., 1992a). The trigeminal nucleus caudalis receives the major synaptic input from the trigeminal nerve and contains neurons that discharge when the meninges are stimulated. When placed in the subarachnoid space, blood is noxious, as evidenced by the development of severe headache, nausea and vomiting, photophobia and vasoconstriction in human. In experimental rats, intraventricular injection of blood increased the number of c-*fos* immunoreactive cell within lamina I and lamina II. Number of responding cells was maximal 2 hours after injection and its number corresponded to the amount of blood injected. Furthermore, inhibition of c-*fos* expression was probably also related to the development of analgesia because pretreatment with morphine decreased the number of expressing cells.

Pretreatment with 5-HT_1 agonist with some selectivity for the B and D subtype receptors reduced the number of c-*fos* positive cells caused by instilled blood. Selective 5-HT_{1D} agonist, sumatriptan or dihydroergotamine decreased the number of positive cells significantly in lamina I and II compound with those in vehicle treated animal (Nozaki et al., 1992b). Sumatriptan did not blocked c-*fos* expression in the trigeminal nucleus caudalis following formalin application to the oral mucosa. This mean that sumatriptan is fundamentally different from such analgesics as morphine.

In theory, therefore, the expression of c-*fos*-like protein could be a specific marker for neuronal activity at the single cell level. Recent reports have described the expression of the c-*fos*-like protein in the dorsal horn of the spinal cord following noxious stimulation and have suggested that the induction c-*fos* may provide a marker for physiological activity (Hunt et al., 1987).

Method of assessing pain

Animal studies on pain employ behavior measures that are of two types: simple withdrawal reflexes and more complex voluntary and intentional behavior that are unlearned or learned (Chapman et al., 1985; Dubner, 1994).

Simple reflex measures

These include the tail-flick test, the limb-withdrawal reflex and the jaw-opening reflex. In most cases latency measures are used to assess reflex responses. In the tail-flick reflex, a radiant heat stimulus is focused on the blackened area of the tail and the animal flicks its tail to escape the stimulus. The technique was introduced by D'Armour and Smith (1941) to demonstrate analgesia. The effectiveness of analgesic agents in this model is highly correlated with their effectiveness in relieving pain in humans (Grumbach, 1966). This model has been used to assess pain produced by brain stimulation, stress or the microinjection of opioids or other chemical mediators (Dubner and Bennett, 1983). In the limb-withdrawal test, thermal or electrical stimuli are typically employed and the latency of a brisk motor response is used as the behavioral endpoint (Bonnett and Peterson, 1975). Limb-withdrawal and tail-flick responses can also be investigated by immersion of the appendage into a waterbath at 47°C (Coderre and Melzack, 1985). The jaw-opening reflex (Mitchell, 1964) is elicited by electrical stimulation of a tooth and electromyographic recordings from jaw muscles are used to assess the behavior. These simple reflex measures permit the animal to have control over stimulus magnitude and thus ensure that the animal can control the level of pain. There are minimal ethical concerns about the use of these measures in conscious animals. The tail-flick reflex has the added advantage that it can be elicited under light anesthesia (Fields et al., 1983; Sandkuhler and Gebhart 1984). The

tail-flick latency is faster in the lightly anesthetized state than in the awake animal.

Reflex responses suffer from a number of limitations as measures of pain behavior (Chapman et al., 1985). They are a measure of reflex activity and not pain sensation. The tail-flick reflex, for example, can be elicited in spinalized animals. Although analgesia in humans and tail-flick latencies are highly correlated, reflex activity in humans produced by noxious stimulation can be dissociated from pain sensation produced by the same stimulus (Willer et al., 1979; McGrath et al., 1981). Changes in reflex activity can result from alterations in motor as well as sensory processing. Drugs that effect motorneuron or muscle function will alter reflex latencies in a manner similar to analgesic drugs. Reflex latencies also are not easily related to stimulus intensity. In fact, most investigators empirically choose a convenient endpoint and normalize distributions to avoid the problem of baseline variability in a large group of animals.

Tissue injury models of persistent pain

The developing model of tissue injury and inflammation that produce responses that mimic human clinical pain conditions in which the pain lasts for longer periods of time. In one test, formalin is injected beneath the footpad of a rat or cat (Duisson and Dennis, 1977; Tjϕlsen et al., 1992). The chemical produces complex response patterns that last for approximately 1 hour. The behavioral state of the animal can be graded numerically ad the effect wears off; initially the animals elevate the limb and do not place it on the cage floor, but during the ensuring 60-90 minutes they begin to use it as a weight-bearing limb.

Models of inflammation that produce more persistent pain include the injection of carrageenan or complete Freund' s adjuvant (CFA) into the footpad (Iadarola et al., 1988) or into the joint of the limb (Schaible et al., 1987). These model result in rapid, short-lasting, acute pain responses, similar in duration to the formalin method; higher doses of the irritants can produce more persistent pain that mimics the time course of postoperative pain or even arthritis. In the inflammation model elicited by injection of CFA into the footpad, the cutaneous inflammation appears within 2 hours and peaks within 6-8 hours. Hyperalgesia and edema are present for approximately 1 week to 10 days. The physiological and biochemical effects are limited to the affected limb (Iadarola et al., 1988a) and there are no signs of systemic disease. It is obvious that the levels of pain are below the tolerance level of the animals. Iadarola et al. (1988b) observed that rats with adjuvant or carrageenan-induced inflammation exhibit minimal reductions in weight and show normal grooming behavior. Exploratory motor behavior is normal and no significant alterations occur in an open-field locomotion test. Thus, the impact of the inflamed limb on the rat's behavior is minimal and the rats will use the limb for support.

Organized learned behaviors

Learned or operant responses are a separate category of behaviors from which pain has been inferred in animals. The most common and simplest method involves an animal escaping a noxious stimulus by initiating a learned behavior such as crossing a barrier of pressing a bar. For example, electric shock can be delivered to a grid floor in a cage and the animal can be trained to jump over a barrier partition to escape the stimulus. The latency of escape is usually measured. Vierck and Cooper, (1984) have developed a sophisticated version of this type of model in monkeys in which there are multiple measures of the animal's escape behavior. Another operant procedure used with electrical stimulation is the shock titration method (Weiss and Laties, 1963). The animals press a bar to reduce the intensity of a continuously increasing stimulus. The animals tend to titrate the stimulus intensity at or near the noxious level. However, this method tends to assess avoidance rather than escape behavior and it is extremely difficult to determine whether the animals are titrating the stimulus in the noxious range or below it.

The more complex methods include reaction time experiments in which the animal detects or discriminates a noxious. In conflict paradigm (Vierck et al., 1971; Dubner et al., 1976), animals learn to perform a task to receive a reward, but also are exposed to noxious stimulation during the task. The animals must choose between receiving a reward or escaping the aversive stimulus on each trial. This method produces escape rather than avoidance behavior in the animals. When monkeys and humans perform such a task with noxious heat stimuli, they exhibit similar escape thresholds (Dubner et al., 1976). Correlative behavioral and neural studies using this model have established that the activity of myelinated mechanothermal nociceptors is sufficient to account for correct performance in such a task (Dubner et al., 1977).

Reaction time tasks have been developed in which monkeys are trained to detect stimuli in the noxious heat range (Dubner, 1985). This task can be designed so that the detection involves stimuli only in the noxious heat range and no cues are provided by preceding innocuous warming stimuli. Another advantage of this behavioral task is that it can be used in conjunction with the detection of innocuous warming stimuli. A comparison of the effects of drug manipulations on innocuous versus noxious stimuli rules out the possibility that drug is altering attentional, motivational or motoric aspects of the animal's behavior instead of influencing the perceived intensity of the noxious stimuli (Oliveras et al., 1986).

As with unlearned behaviors related to pain, all of the above operant procedures provide only indirect measures of pain such as the latency or the probability of a motor response. However, they have the advantage over simpler unlearned behaviors in that the magnitude of the behavioral change varies with stimulus intensity, providing more reliable evidence that the change in behavioral change in behavior reflects the perception of a noxious stimulus rather than a change in motor performance. Sophisticated operant tasks in animals also allow the experimenter to rule out that changes in performance are related to attentional and motivational variables rather than changes in sensory perception. It also should be recalled that these operant procedures give the animal control over the stimulus and other parameters of the experiment to a degree comparable to that found in human studies of experimental pain.

The Purpose of the Study

Evidence from various sources indicated that serotonin receptor types I and II might produce algesic or analgesic effects. In order to clarify this hypothesis, we performed a series of experiments in rat to study the role of serotonin receptor types I and II in pain modulation.

The aims of this study are:

1. To study the effects of 5-HT_1-agonist and 5-HT_2-agonist on modulation of nociceptive system by measuring tail flick latency.
2. To study the effects of 5-HT_1-agonist and 5-HT_2-agonist on modulation of nociceptive system by observing formalin-induced pain behavior response.
3. To study the effects of 5-HT_1-agonist and 5-HT_2-agonist on modulation of nociceptive system by using c-fos expression to evaluate pain intensity.