CHAPTER I

INTRODUCTION

Significance of Problem

Since its introduction into clinical medicine one century ago (Von Meyring 1893; Hanks, 1983), paracetamol (acetaminophen) has been extensively used for the treatment of pain and fever. Its therapeutic mechanism of action is still unclear and it is still not known whether paracetamol acts peripherally, centrally or both.

Paracetamol possesses analgesic activity against pain of mild to moderate severity but has few anti-inflammatory properties and exerts its analgesic effect via a central action. The explanations for these properties were based on the greater ability of paracetamol in inhibiting the isoforms of cyclooxygenase present in the central nervous system rather than those present peripherally (Flower and Vane, 1972; Dembinska-kiec et al., 1976; Brucchausen and Baumann, 1982). As a conformation of these *in vitro* studies, clinical experiments have shown that therapeutic doses of paracetamol did not reduce prostaglandins type $F_{2\alpha}$ ($PGF_{2\alpha}$) urinary excretion (Bippi and Frohich, 1990). There is some evidence that prostaglandins (PGs) are involved in the central modulation of pain (Ferreira et al., 1978; Chapman and Dickenson, 1992; Malmberg and Yaksh, 1992), and it has been suggested that the degree of inhibition of brain cyclooxygenase correlated well with the antinociceptive potencies of NSAIDs

(Ferreira et al., 1990; Malmberg and Yaksh, 1992). All these data would suggest that the specific pharmacological profile and the analgesic property of paracetamol might be due to a central mechanism involving an inhibitory effect central cyclooxygenase.

Evidence of a central mechanism, widely described for NSAIDs in several reviews (Brune, 1994; Mc Cormack, 1994; Bannwarth et al., 1995), exists for paracetamol. Its physicochemical properties (pKa of 9.5 and thus a largely unionized form in the physiological range of pH) (Forrest et al., 1982) and its pharmacokinetic characteristics, such as its weak binding to plasma proteins (Gazzard et al., 1973; Clissold, 1986) or its ability to cross the human and rat blood brain barrier (Ochs et al., 1985; Bannwarth et al., 1992), are compatible with a central effect. Several experimental (Hunskaar et al., 1985; Carlsson et al., 1988; Gropetti et al., 1988; Jurna and Brune, 1990) and clinical (Chen and Chapman, 1980; Bromm et al., 1991; Piletta et al., 1991) studies have demonstrated such an effect. However, whether paracetamol, a weak inhibitor of PG synthesis (Brune et al., 1991), selectively acts on central nervous system cyclooxygenases is questionable (Vane, 1971; Tolman et al., 1983; Lanz et al., 1986). This has lead to study other hypotheses for its mechanism of action. Hunskaar et al.(1985) reported that paracetamol inhibited spinally substance P (SP)-mediated hyperalgesia, suggesting that paracetamol-induced analgesia may be related to modulation of nociceptive transmission in spinal and supraspinal pathways. This observation was particularly interesting in light of recent evidence demonstrating that paracetamol reduced nitric oxide generation involved in spinal hyperalgesia induced by N-methyl-D-aspartate (NMDA) and substance P (Bjorkman et al., 1994). Interestingly, Piletta et al.(1991) showed that paracetamol, unlike aspirin, was able to induce in humans a centrally mediated analgesia, using the method based on the

nociceptive flexion reflex of the leg (RIII-reflex), previously described by Willer and Harrewyn. Inaddition, it has been shown that lesioning of bulbospinal serotonergic pathways with 5,6-dihydroxytryptamine in rats reduced the antinociceptive effect of paracetamol (Tjølsen et al., 1991).

Surprisingly, other authors have demonstrated that this compound has only a weak antinociceptive effect when injected intracerebroventricularly (i.c.v.) in rats (Okuyama and Aihara, 1984). This lack of activity may result from its inability to inhibit prostaglandin synthesis in rat CNS tissue (Abdel-Halim et al., 1978).

It has been suggested that central monoaminergic and serotonergic pathways may be involved in pain modulation (Shyu and Lin, 1985; Warner et al., 1990) and that there may be a connection between analgesia induced by some NSAIDs and the increase in the turnover rate of dopamine, noradrenaline and serotonin (5-HT) in the rat CNS (Bensemana and Gascon, 1978; Groppetti et al., 1988). Moreover, prostaglandins seem to interact with a descending modulating system by inhibiting the release of neurotransmitters in the spinal cord (Taiwa and Levine, 1988).

Many types of analgesic drugs (morphine, NSAIDs) have been proposed to act through an increase in brain serotonin level. The monoaminergic pathways have been shown to be crucial in the antinociceptive mechanisms, for they modulate the noxious stimuli at various levels of the CNS (Sandrini et al., 1986; Pascual, 1990; Kobal et al., 1990; Wang and Teng, 1991). The serotonergic system may regulate nociception in different ways, depending on the receptor subtypes involved and serotonin has been claimed to exert its central nociceptive effect in defined brain areas through its receptor subtypes, notably 5-HT_{1A} (Eide and Hole, 1991), 5-HT_2 (Meller et al., 1991;

Alheider, 1991) and 5-HT_3 at the spinal level (Pelissier et al., 1995). Moreover, the bulk of data suggested that stimulation of 5-HT_1 receptor reduced nociceptive sensitivity, whereas activation of 5-HT_2 receptors increased nociceptive responsiveness (Eide and Hole, 1993).

The previous observation suggested that the antinociceptive effect of phenazone and acetylsalicylic acid (ASA) in the hot plate test in rats was associated with a decrease in the number of serotonin receptors in certain brain areas (Sandrini et al., 1993; Pini et al., 1995). Moreover, the pretreatment with parachlorophenylalanine (PCPA) prevented the reduction of the number of 5-HT receptors induced by ASA or phenazone and abolished the analgesic activity on the hot plate test (Pini et al., 1993). These data suggested that paracetamol exerted its antinociceptive effect through the 5-HT system.

Blood platelets are believed to be an excellent model for the pre- and postsynaptic 5-HT neurons. Their respective cell membrane share similar supplies of receptors (Malmgren and Hasselmark, 1989). Accordingly, platelet 5-HT has been studied extensively in various diseases including migraine (D'Andrea et al., 1995). However, the information about changes in platelet 5-HT in chronic use of paracetamol is quite limited. A significant increase of 5-HT level in pons and cortex was reported in acute administration of paracetamol in experimental animals (Pini et al., 1996). Moreover, it was shown that chronic use of paracetamol could deplete platelet 5-HT in migraine patients (Srikiatkhachorn and Anthony, 1996).

Review of Serotonin

Serotonin (5-HT) is a neurotransmitter involved in a large number of psycho-physiological processes including the regulation of mood, arousal, aggression, sleep, learning, nociceptions, nerve growth and appetitive functions. Alterations of 5-HT have been shown to occur in many psychiatric diseases including depression, anxiety, eating disorders, schizophrenia, migraine etc. (see review of Jacobs and Azmita, 1990).

Tissue stores of 5-HT

5-HT is found in the following locations :

(1) Intestine : About 90% (10 mg) is located in the enterochromaffin cells of the intestinal tract, 10% of the remaining 5-HT is also found in the myenteric plexus of the intestine, where it is believed to function as an excitatory neurotransmitter mediated enhanced gastric motility.

(2) Blood : 5-HT is present in high concentrations in platelets, which acquire it from plasma. The platelets release 5-HT when they aggregate at a site of vessel wall injury (platelet release reaction). Its functional effects at this site are mostly additive to those of thromboxane A_2, a product of cyclo-oxygenase activity with vasoconstrictor activity which is released from platelets during the initial phases of vessel wall injury.

(3) Central nervous system : 5-HT is also present in the brain, and is concentrated particularly in the midbrain areas and spinal cord. Its function of these sites is discussed below.

Biosynthesis and metabolism of 5-HT

5-HT is synthesized from the amino acid precursor tryptophan which is derived from dietary sources. The dietary amino acid tryptophan is converted to 5-hydroxy-tryptophan (5-HTP) by the enzyme tryptophan hydroxylase (the rate limiting enzyme). This enzyme is found only in cells that synthesize 5-HT. 5-HTP decarboxylase then converted this intermediate amino acid to 5-HT. 5-HT is metabolized both pre- and postsynaptically by the enzyme monoamine oxidase (MAO), which produces the inactive metabolite 5-hydroxyindoleacetic acid (5-HIAA) The synthesis and primary metabolic pathways of 5-HT are shown in Figure 1.

Physiological Pathway of 5-HT

After synthesis, 5-HT is stored in synaptic vesicles via an ATP-dependent, low affinity transport system. Depolarization of the presynaptic endplate membrane induces Ca^{2+} influx, which in turn triggers the release of 5-HT, possibly by activation of intracellular microtubules (Mulder et al., 1975). The excreted 5-HT diffuses to the postsynaptic membrane and binds to and activates postsynaptic receptors. Concomitantly, 5-HT is taken up into the presynaptic neuron to terminate the activation of postsynaptic receptors and relieve 5-HT synthesis. The relevance of reuptake for homeostasis of 5-HT can be derived from the high efficacy of 5-HT recycling. Serotonin is translocated into the presynaptic neuron via an active Na^+-dependent transport system. Following reuptake it is stored in synaptic vesicles by a reserpine-sensitive, H^+-dependent, active transport system and is available for release again (Rudnick, 1986). It has been postulated that the Na^+-dependent -5-HT -reuptake system is functionally associated with the hypothetical presynaptic 5-HT autoreceptor

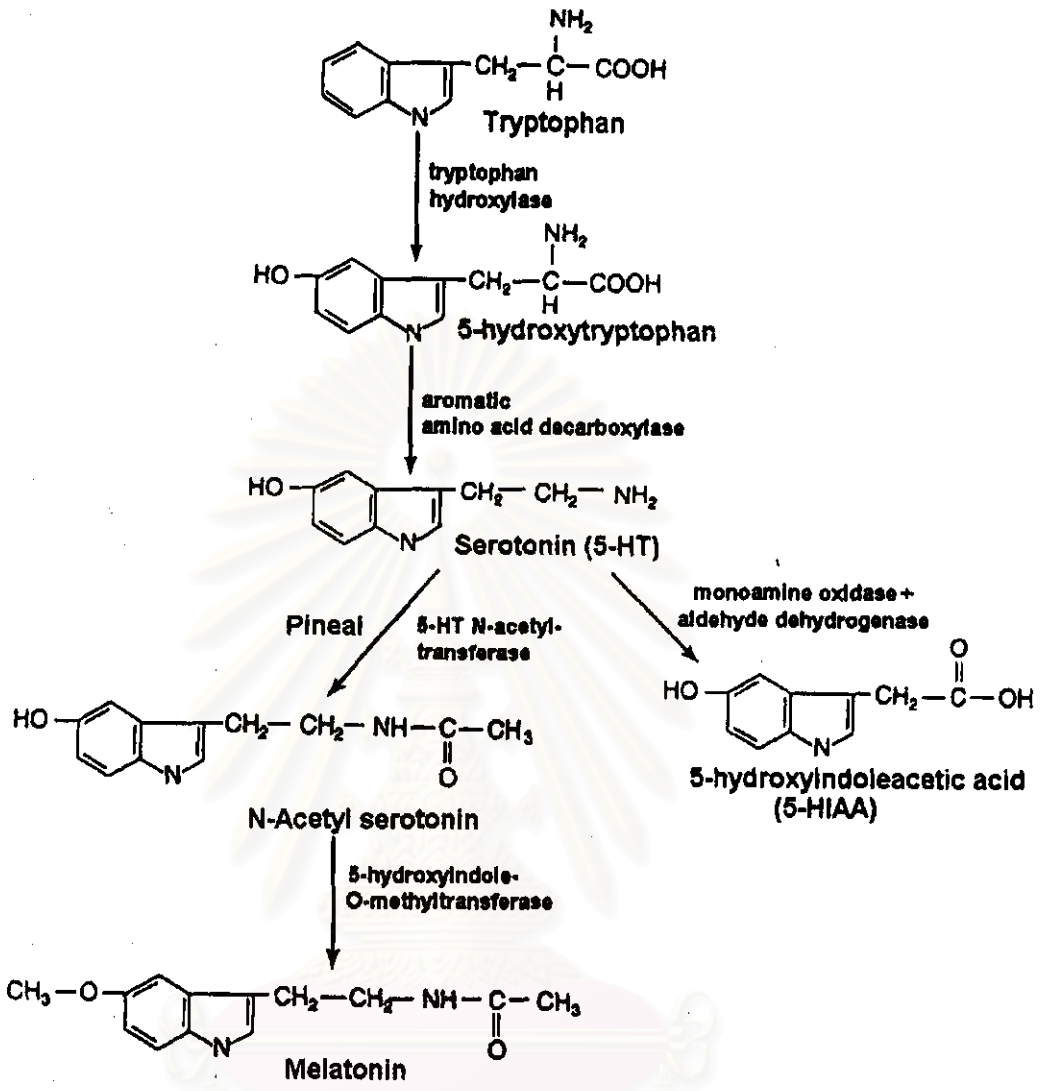

Figure 1. The biosynthesis and catabolism of serotonin. (Siegel GJ et al., 1994).

(Bonnano and Raiteri, 1987; Galsin et al., 1985) and, as such, is involved in feedback processes regulated 5-HT synthesis and release (Fig.2).

Anatomical Distribution of Serotonin

The original mapping of 5-HT neurons was done by Dahlstrom and Fuxe (1964) in the rat using the Falck histofluorescence technique. In addition to the initial mapping of Dahlstrom and Fuxe, the distribution of 5-HT neurons has been determined in the rat using immunohistochemistry for tryptophan hydroxylase (Tr-OH) (Pickel et al., 1976). Immunohistochemistry for 5-HT has been used for mapping in the rat (Steinbusch, 1981, 1984; Lidof et al., 1980).

The 5-HT cell bodies in the brain are located in the brain stem on or near the midline. Their axons, however, innervate nearly every area of the central nervous system (Fig 9). In 1964, Dahlstrom and Fuxe observed that the majority of serotonergic soma was found in cell body groups previously designated by Taber, Bradal and Walberg as the raphe nuclei. This earlier description of the raphe nuclei was based on cytoarchitectural criteria, i.e., on cell body structural characteristics and organization. Dahlstrom and Fuxe described nine groups of serotonin-containing cell bodies, which they designated B_1 through B_9, and which correspond for the most part with the raphe nuclei. Some serotonergic neuronal cell bodies, however, are found outside the raphe nuclei are serotonergic.

The largest group of serotonergic cells is group B_7 of Dahlstrom and Fuxe. Group B_7 is contiguous with a smaller group of serotonergic cells, B_6. Groups B_6 and B_7 are often considered together as the dorsal raphe (DR) nucleus, with B_6 being

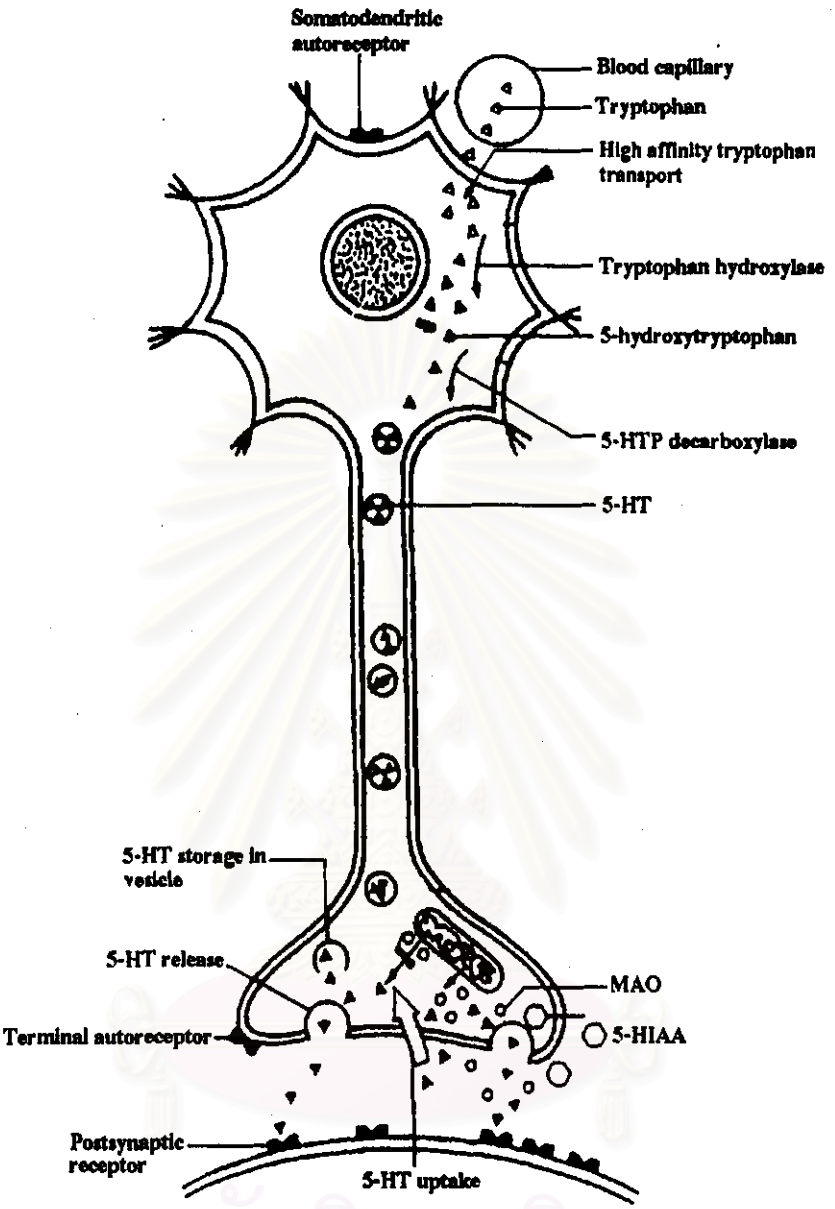

Figure 2. Schematic diagram of serotonergic neuron. 5-HT is synthesized by transporting tryptophan from bood into brain. After synthesis, 5-HT is stored in vesicle. Depolarization triggers 5-HT release to bind to post-synaptic receptor. Concomitantly, 5-HT is taken up into presynaptic neuron and metabolized to 5-HIAA by monoamine oxidase (MAO). (Feighner JB and Boyer WF, 1991).

its caudal extension. Another prominent serotonergic cell body group is B_8, which corresponds to the median raphe (MR) nucleus, also termed the nucleus centralis superior. Group B_9, part of the ventrolateral tegmentum of the pons and midbrain, forms a lateral extension of the median raphe and therefore is not considered one of the midline raphe nuclei. Ascending serotonergic projections innervating the cerebral cortex and other regions of the forebrain come from the dorsal raphe, median raphe, and B_9 cell group. The other raphe nuclei, B_1 to B_5, are more caudally situated (mid pons to caudal medulla) and contain a small number of serotonergic cells. These cell body groups give rise to serotonergic axons that project within the brain stem and to the spinal cord.

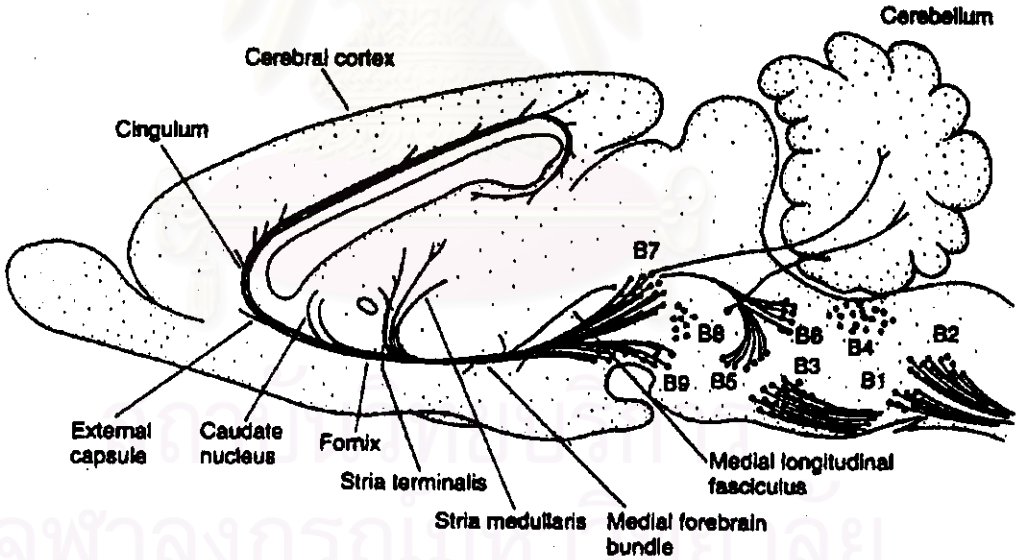

Figure 3. Schematic drawing depicting the location of the serotonergic cell body groups in a sagittal section of the rat central nervous system and their major projections (Kandel ER and Schwartz JH, 1991).

The highest percentage of 5-HT is located in the DR (79%) and the bulk of the remainder produced by the MR. Thus the main source for limbic and neocortical 5-HT is DR and MR nuclei (Azamita,1978; Azamita and Gannon, 1986; Wilson and Molliver, 1991a,b). Specifically, the amygdala, hypothalamus, basal ganglia, primary and association receiving area, and frontal lobe are innervated by the DR, whereas the hippocampus, cingulate gyrus, and septum receive their 5-HT from the MR nuclei (Azamita,1978; Azamita and Gannon, 1986; Wilson and Molliver, 1991a,b).

The MR, however, is diffusely organized and appears to exert a nonspecific and global influence on arousal and excitability (Wilson and Molliver,1991a,b). The DR is much more discretely organized, can exert highly selective inhibitory or excitatory influences, plays a role in the coordination of excitation in multiple functionally related areas, including the frontal lobes and amygdala (Wilson and Molliver,1991a,b). Because of the manner in which they are organized, the DR and MR can exert select inhibitory influences so as to engage in perceptual filtering in one or a variety of areas.

In summary, it can be said that the number of 5-HT cell bodies in the brain are remarkably few, with the overwhelming majority being in the raphe and reticular systems. Newer techniques, especially the immunocytochemical technique for 5-HT, have confirmed the main outlines of the original distribution of 5-HT neurons described by Dahlstrom and Fuxe with a number of significant additions, particularly in terms of the spread of brain stem 5-HT groups laterally from the midline confines of the raphe nuclei. Some species differences may exist, which account for minor discrepancies reported among the rat, cat, and monkey, such as the area postrema.

Serotonin Pathways and Terminals

While the number of 5-HT cell bodies in the CNS are considerably fewer than for the catecholamines, terminals are equally ubiquitous. Every area of brain and spinal cord receives 5-HT nerve endings. The more caudal 5-HT cells project particularly to the spinal cord, while the more rostral ones project to the diencephalon and forebrain. Projections are all over-whelmingly ipsilateral. Double labeling experiments have generally established extensive overlap between cell body areas and terminal fields. Therefore, the main fiber bundles represent a mixture of neuronal groups of origin.

Pathways to the spinal cord are intermediate, ventral, and dorsal. The intermediate descending bulbospinal pathway arises mainly from the nuclei raphe pallidus and obscurus. It projects through the intermediate zone and terminates in the intermediate lateral cell column. The ventral descending bulbospinal pathway also arises from the nuclei raphe pallidus and obscurus and terminates in the region of anterior horn cells (R.F. Martin et al.,1978). The dorsal descending pathway travels down the dorsolateral funiculus to reach the dorsal horn. It contains only a minority of serotonergic fibers.

Bowker et al. (1981) have shown that medullary cell groups B_1-B_3, as well as B_5, provide the major 5-HT input to the entire spinal cord, while cell groups B_7 and B_9 of the midbrain project to the cervical and probably the upper thoracic spinal cord. The densest innervation is to the substantia gelatinosa.

Ascending projections are dorsal, medial, and ventral. The dorsal ascending pathway arises primarily from the medial and rostral parts of the nucleus raphe

dorsalis. It runs dorsolateral to the medial forebrain bundle and terminates predominantly in the neostriatum. The medial ascending pathway originates largely in the raphe dorsalis and projects primarily to the substantia nigra. The ventral ascending pathway is the major pathway. It innervates the diencephalon, limbic system, and cortex.

5-HT Receptors

The known effects of 5-HT in the brain and the periphery are thought to be mediated by the interaction between serotonin and receptors on the cell surface of sensitive cells. Finding over the last 10 years have shown that 5-HT acts not through a single receptor, but through a myriad of different receptor types. These actions, though mediated through a number of receptors, all feed into one of several signalling pathways common to many cell types. These pathways include control of the levels of cyclic AMP (cAMP), control of the production of inositol phosphates, release of arachidonic acid, or the direct control of membrane conductances through the direct gating of an ion channel intrinsic to the receptor itself.

In the relatively few years that have passed since Bradley et al.(1986) proposed the first unifying scheme for naming and classifying 5-HT receptors, 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). Obviously, the molecular properties of a receptor provide fundamental information of identification, but its

recognitory 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-$HT_{1D\beta}$ 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).

Against this background, the 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; its *operational* (drug-related), *transductional* (receptor-coupling) and *structural* (primary amino acid sequence) characteristics (Humphrey et al., 1993; Hoyer et al., 1994). When applied to the currently recognized 5-HT receptors, these criteria indicate the existence of up to seven receptor classes and provide a rational basis for the relocation of, for example, the 5-HT_{1C} receptor to the 5-HT_2 class. Importantly, they also assist in the classification of new recombinant receptors. Table 1 review the characteristics of the principal 5-HT receptor class. The later section describes, briefly, the different 5-HT receptor subtypes.

Radioligand binding studies originally provided evidence for two distinct 5-HT receptor subtypes in the brain, 5-HT_1 and 5-HT_2 (Peroutka and Snyder, 1979). Based on more recent pharmacological and biochemical criteria, four major 5-HT receptor families, termed 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 (Bradley et al., 1986; Hoyer and Schoeffter, 1991) have been identified, with each family possibly comprising numerous receptor subtypes (Peroutka, 1988). In particular, the 5-HT_1 receptor family has been subdivided into five receptor populations, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F} (Leonhardt et al., 1989; Schmidt and Peroutka, 1989; Hartig et al., 1990;

Table 1. Current proposals for classifying and naming 5-HT receptors based on an integrations of operational, transductional and structural information

Receptor name	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1E}	5-HT_{1F}
Previous name	—	—	—	—	$5\text{-HT}_{1E\beta}$, 5-HT_{6}
Selective agonists	8-OH-DPAT	CP93129	sumatriptan LY694,247	—	—
Selective antagonists (pK_B)	(±) WAY 100135 (7.2-7.7)	—	GR127935	—	—
Radioligands	$[^3H]$-8-OH-DPAT	$[^{125}I]$-GTI	$[^{125}I]$-GTI	$^3[^3H]$-5-HT	$^3[^{125}I]$LSD
Effector pathways	↓ cAMP K^+ channel	↓ cAMP	↓ cAMP	↓ cAMP	↓ cAMP
Gene	5-ht_{1A}	5-ht_{1B}	5-ht_{1D}($5\text{-ht}_{1D\alpha}$*human*) 5-ht_{1B}($5\text{-ht}_{1D\beta}$*human*)	5-ht_{1E}	5-ht_{1F}
Structural information	421 aa *human* 7TM 422 aa *rat* 7TM	386 aa *rat* 7TM 386 aa *mouse* 7TM	377 aa *human* 7TM 390 aa *human* 7TM	365 aa *human* 7TM	366 aa *human* 7TM 366 aa *rat* 7TM
Receptor name	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_{3}	5-HT_{4}
Previous name	D, 5-HT_2	5-HT_{2F}	5-HT_{1C}	M	—
Selective agonists	α-Me-5-HT	α-Me-5-HT	α-Me-5-HT	2-Me-5-HT m-chlorophenyl-biguanide	5-MeOT renzapride BIMU 8
Selective antagonists (pK_B)	ketanserin (9.2) ritanserin (9.5) LY 53857 (8-9.5)	[2]LY53857 (~8.0)	mesulergine (9.1) LY53857 (8.0-9.5)	tropisetron (10-11) ondansetron (8-10) granisetron (10)	GR113808 (9.0-9.5 SB204070 (10.8)
Radioligands	$[^3H]$-ketanserin	$^3[^3H]$-5-HT	$[^3H]$-mesulergine	$[^3H]$-zacopride	$[^3H]$-GR113808
Effector pathways	IP_3/DAG	IP_3/DAG	IP_3/DAG	ligand-gated cation channel	↑cAMP
Gene	5-ht_{2A}	5-ht_{2B}	5-ht_{2C}	$5\text{-ht}_{3R\alpha}$	—
Structural information	471 aa *human* 7TM 471 aa *rat* 7TM	479 aa *rat* 7TM 504 aa *mouse* 7TM	458 aa *human* 7TM 460 aa *rat* 7TM	487 aa *mouse* ion channel unit	—
Receptor name	[4]5-ht_{5A}	[4]5-ht_{5B}	[5]5-ht_{4}	[6]5-ht_{7}	
Previous name	$5\text{-HT}_{5\alpha}$	$5\text{-HT}_{5\beta}$	—	—	
Selective agonists	—	—	—	—	
Selective antagonists (pK_B)	—	—	—	—	
Radioligands	$^3[^{125}I]$LSD	$^3[^{125}I]$LSD	$^3[^{125}I]$LSD $^3[^3H]$5-CT	$^3[^{125}I]$LSD $^3[^3H]$-5-HT $^3[^3H]$-5-CT	
Effector pathways	Unknown	Unknown	↑cAMP	↑cAMP	
Gene	5-ht_{5A}	5-ht_{5B}	5-ht_{6}	5-ht_{7}	
Structural information	357 aa *mouse, rat* 7TM	370 aa *mouse* 7TM 371 aa *rat* 7TM	436 aa *rat* 7TM	448 aa *mouse, rat* 7TM 466 aa *g-pig* 7TM 445 aa *human* 7TM	

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[1]The 5-HT_{1B} receptor is the rat homologue of the human $5\text{-HT}_{1D\beta}$ receptor

[2]unsurmountable antagonism.

[3]in cloned cells.

[4]Erlander et al., 1993; Matthes et al., 1993.

[5]Ruat et al., 1993a; Monsma et al., 1993.

[6]Shen et al., 1993; Ruat et al., 1993b; Meyerhof et al., 1993; Lovenberg et al., 1993; Tsou et al., 1994.

Palacios et al., 1990; Hoyer and Schoeffter, 1991), primarily on the basis of their distinct pharmacological profiles and tissue distribution within the CNS. Of the numerous 5-HT receptors identified, five have been cloned and pharmacologically characterized : 5-HT_{1A} (Fargin et al., 1988; Kobilka et al., 1987; Albert et al., 1990), 5-HT_{1B} (Branchek et al., 1991), 5-HT_{1C} (Julius et al., 1988), 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F} (Hamblin and Metcalf, 1991), and 5-HT_2 (Pritchett et al., 1988; Julius et al., 1990). These belong to a large group of neurotransmitter and peptide hormone receptors whose biological effects are mediated via G proteins. The 5-HT_3 receptor is unique among 5-HT receptors because it appears to belong to the ligand-gated ion channel superfamily. (Yankel and Jackson, 1988). The recent pharmacologically characterized 5-HT_4 or 5-HT_4-like receptors (Dumuis et al., 1988; Kaumann, 1990) that mediate vascular relaxation appear to be G protein-coupled receptors associated with stimulation of adenylyl cyclase. The 5-HT_4 receptor has not been cloned yet.

The 5-HT_{5A} and 5-HT_{5B} receptors recently have been added to the 5-HT receptor family (Erlander et al., 1993; Matthes et al., 1993; Plassat et al., 1992). The pharmacological profiles of the mouse 5-HT_{5A} and 5-HT_{5B} receptors are similar, with low affinity for 5-HT, but high affinity for 5-CT and a low affinity for sumatriptan. The 5-HT_{5A} and 5-HT_{5B} receptors share 77% sequence similarity, and both genes contain introns. Coupling of 5-HT_{5A} to either adenylyl cyclase or phospholipase C has not been detected, and it is possible that both receptors couple to ion channels.

Another recent addition to the serotonin family is a novel 5-HT receptor with high affinity for tricyclic psychotropic drugs (Monsma et al., 1993). This receptor has been designated a 5-HT_6 receptor and is a 437 amino acid G protein-coupled receptor. It has been cloned from rat striatum, and it exhibits low (<50%) identity to previously

cloned 5-HT receptors. The 5-HT_6 receptor mRNA localizes to limbic and cortical brain regions, suggesting its possible involvement in neuropsychiatric disorders.

The newest member to date in the serotonin family is 5-HT_7 receptor (Ruat et al., 1993; Shen et al., 1993). Although this receptor displays high affinity for serotonin, it is clearly distinguishable by its pharmacology from the 5-HT_1 receptors, and it couples positively to adenylyl cyclase. The presence of introns is also a characteristic of this receptor, which seems to be found associated with limbic brain areas.

The rich diversity of 5-HT receptor subtypes makes it important to elucidate the structure and regulation of individual receptor subtypes so that animal models for mental disorders in which serotonin is involved can be developed, and new subtype-specific drugs can be discovered and evaluated.

Because this study involved the effect of paracetamol on central 5-HT_{2A} receptor, thus, the characteristics of 5-HT_2 receptors were reviewed in more detail.

The 5-HT_2 Receptors

The 5-HT_2 receptor class now comprises three distinct receptor subtypes, namely 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}. Each is a seven transmembrane, G-protein-linked receptor similar in molecular size and displaying about 60% sequence homology. In contrast to 5-HT_1 receptors, 5-HT_2 receptor genes possess introns and exons and are coupled preferentially to phospholipase C. Receptor activation therefore leads to stimulation of phosphatidyl inositol metabolism and an increase in inositol triphosphate (IP_3) production.

Localization of the 5-HT$_2$ Receptors

The 5-HT$_2$ receptor has been identified in a variety of tissues, both in the peripheral and central nervous systems. In the periphery, this receptor has been localized to several smooth muscle types including the guinea pig trachea and rat uterus. In these tissues stimulation with 5-HT$_2$ receptor agonists causes a contraction of the smooth muscle (Heller and Baraban, 1987; Cohen et al., 1985). In the central nervous system, the distribution of 5-HT$_2$ receptor has been extensively mapped, initially with radioligand receptor autoradiography and RNA blot analysis and, more recently, with immunohistochemistry.

Radioligand autoradiography with [^{3}H]ketanserin in rat brain revealed high concentrations of the 5-HT$_2$ receptor in the claustrum, olfactory tubercle, Layers I and V of the neo-cortex, pyriform cortex, and anterior olfactory nucleus (Pazos et al.,1987). Lesser concentrations of receptor were identified in caudate, putamen, nucleus accumbens, Layer V of cortex, ventral dentate gyrus, and mammillary bodies. Little to no 5-HT$_2$ receptor labeling has been observed in thalamus, hippocampus, brain stem, medulla, cerebellum, and spinal cord (Pazos et al.,1987).

RNA blot analysis clearly shows that 5-HT$_2$ receptor mRNA is expressed predominantly in the cerebral cortex (Julius et al., 1990). Transcripts are also seen in the hypothalamus, hippocampus, spinal cord, and olfactory bulb, but at about ten-fold lower abundance. This is consistent with the distribution of 5-HT$_2$ binding sites described for the rat brain (Pazos et al., 1985). Thus, whereas the 5-HT$_2$ receptor predominates in the cerebral cortex, the 5-HT$_{2C}$ receptor is more widely expressed and far more abundant in the CNS.

5-HT$_{2A}$ receptors

The newly named 5-HT$_{2A}$ subtype refers to the 'classical' 5-HT$_2$ receptor widely described in peripheral tissues and the CNS. In the periphery, activation of the receptor leads to contraction of vascular and non-vascular smooth muscle, platelet aggregation and increase in capillary permeability. Available evidence also implies a role in modulating the release of other neurotransmitters and hormones, including acetylcholine (Muramatsu et al., 1988a), adrenaline (Feniuk et al., 1981), dopamine (Muramatsu et al., 1988b), excitatory amino acids (Maura et al., 1988), and vasopressin (Rittenhouse et al., 1990). In the CNS, autoradiographic studies reveal highest receptor concentrations in the cortex and to a lesser extent in hippocampus and caudate nuclei (Hoyer et al., 1986; Pazos et al., 1987). Precise roles in the CNS remain unclear, but in rodents agonists acting at 5-HT$_{2A}$ receptors evoke a stereotypical syndrome comprising head twitch and wet-dog shakes, implying a possible involvement of the receptors in motor behaviours. An important role in regulating sleep has been suggested (Sharpley et al., 1990). Furthermore, in peripheral (e.g. rat facial and spinal motoneurones: Connell and Wallis, 1989) as well as central (e.g. nucleus accumbens: North and Uchimura, 1989) neurones, 5-HT$_{2A}$ receptors mediate neuroexcitation and have been associated with the transmission of nociceptive stimuli.

Operational Characteristics

A wide range of potent 5-HT$_{2A}$ receptor antagonists are available and several are useful radiolabelled probes, e.g. [^{3}H]spiperone, [^{3}H]ketanserin, [^{3}H]mesulergine, [^{3}H]mianserin, [^{3}H]lysergic acid diethylamide ([^{3}H]LSD), [^{3}H]DOB and more

recently [^{3}H]RP62203 (Mylecharane, 1990; Malgouris et al., 1993). Unfortunately, all display poor selectivity with regard to the three 5-HT_2 receptor subtypes, and none alone can be relied upon to define the 5-HT_{2A} receptor unequivocally. Among the most useful antagonists are ketanserin and RP62203, both of which display selectivity over 5-HT_{2C} receptors.

Several specific antagonists exist to separate 5-HT_2 effects from other 5-HT receptors. Spiperone, for example, can distinguish between the 5-HT_{1C} receptors and the 5-HT_2 receptors with 1000-fold difference in affinity. But it has a fairly high affinity for the 5-HT_{1A} receptor, and an even higher affinity for dopamine D_2 receptors. Ketanserin has approximately 100-fold difference in affinity for the 5-HT_2 receptor than for any of the other 5-HT receptors, but it has appreciable affinity for both histamine H_1 receptors and α_1-adrenergic receptors. DOB and DOI have been used as selective agonists for 5-HT_2 receptors, though both compounds have some affinity for the 5-HT_{1C} receptors and some caution must be used in studies using these compounds (Leysen, 1989). There are no subtype selective 5-HT_2 receptor agonists. α-Methyl-5-HT and the phenylalkylamines (DOI, DOB) are most often used in this regard, but are equally active at 5-HT_{2A} , 5-HT_{2B} and 5-HT_{2C} receptors and, in addition, are potent agonists at an orphan 5-HT receptor mediating endothelium-dependent vasorelaxation (Martin, 1994).

It is important to recognize that antagonist affinity estimates at the 5-HT_{2A} receptor may vary considerably, but there is presently no evidence to suggest that this reflects the existence of 5-HT_{2A} subtypes. Rather, it appears to result from species differences in receptor primary structure. However, binding data obtained using [^{3}H]DOB and [^{3}H]ketanserin reveal intriguing differences indicating that either the

receptor exists in two non-interconvertible states or that post-translational modifications have yielded two closely similar forms of the receptor (Branchek et al., 1990).

Transductional characteristics

Receptor coupling to IP_3 production has been demonstrated in numerous studies using either native or recombinant 5-HT_{2A} receptors. However, Felder et al. (1990) have shown that 5-HT can stimulate phospholipase A_2 and subsequent release of arachidonate in hippocampal and cortical neurones via 5-HT_2 receptors presumed to be 5-HT_{2A} .

Unlike the 5-HT_1 receptors, agonist activation of the 5-HT_2 receptor results in phospholipid hydrolysis and production of the second messenger phosphoinositide. Conn and Sanders-Bush (1985) demonstrated a 5-HT-mediated increase in phosphoinositide turnover in rat cortical slices that could be blocked by ketanserin. This effect is diminished in cortical slices from animals chronically treated with imipramine and iprindole (Kendall and Nohorski, 1985). As would be expected of a receptor linked to phosphoinositide hydrolysis, stimulation of the 5-HT_2 receptor causes translocation of protein kinase C from the cytosol to the membrane as part of the second messenger cascade (Wang and Friedman, 1989).

Structural characteristics

Human (Saltzman et al., 1991), rat (Pritchett et al., 1988), mouse (Yang et al., 1991) and hamster (Chambard et al., 1990) 5-HT_{2A} receptor genes have now been cloned and each corresponding receptor comprises 471 amino acids. Human-rat sequence homology is 91% overall, and 99% in the transmembrane region. Serine[242]

in the fifth transmembrane region of the human receptor appears to be an important determinant of ligand binding, since mutation to alanine, as found in the rat sequence, converts the operational characteristics of the human 5-HT_{2A} receptor to that of the rat (Hartig et al., 1990; Kao et al., 1992).

Rat 5-HT_{2A} receptors

5-HT_2 receptor cDNA was isolated from a cloned cDNA library (Pritchett et al., 1988) using probes generated from the 5-HT_{1C} receptor amino acid sequence. The rat 5-HT_{2A} receptor is composed of 471 amino acids, not 449 amino acids as reported initially (Pritchett et al., 1988). Fifty percent of the amino acid sequence is identical to the sequence encoding the 5-HT_{1C} receptor. Radioligand binding on membranes from a transfected mammalian cell line reveals high affinity binding with [^{3}H]spiperone, 5-HT antagonists, such as ketanserin and mianserin, potently (K_i = 1-2 nM) inhibited [^{3}H]spiperone binding to transfected cell membranes. In contrast, the 5-HT_{1A} agonist, 8-OH-DPAT, and the 5-HT_3 antagonist, MDL 72222, displayed low affinity for [^{3}H]spiperone binding. 5-HT was reported to increase intracellular Ca^{2+} and activated phosphoinositide hydrolysis in the transfected cell line. These observations have been confirmed and extended by other laboratories (Julius et al., 1990; Apud et al., 1992).

Radioligand data from a cell line containing expressed rat 5-HT_2 receptors indicate that both [^{3}H]ketanserin and 4-bromo-2,5-[^{3}H]dimethoxyphenylisopropylamine ([^{3}H]DOB) binding sites can be detected (Branchek et al., 1990; Teitler et al., 1990). The [^{3}H]DOB binding sites represent only approximately 30% of the density of the [^{3}H]ketanserin-labeled binding sites (Branchek et al., 1990). These data

seem to suggest two major possibilities: either the 5-HT_2 receptor in membrane preparations exists in at least two distinct, but noninter-converting, states that differ in their binding characteristics, or posttranslational receptor changes may result in two slightly different forms of the 5-HT_2 receptor.

5-HT_{2B} Receptors

This receptor type was originally described as 5-HT_{2F} after its gene cloned and the receptor shown to display the pharmacolgical properties of the receptor mediating contraction of the rat stomach fundus (Kursar et al., 1992; Wainscott et al., 1993). Other functional effects have not been demonstrated, although mRNA has been shown to be present in rat heart, lung, kidney, GI tract and discrete regions of the brain (Foguet et al., 1992).

Serotonin and Pain

5-HT can influence pain mechanisms in several ways. In peripheral tissues, release of 5-HT may be induced by mechanical injury, noxious heat, radiation, or by the action of co-products of tissue damage such as thrombin, collagen and adrenaline. This may have implications for peripheral nociceptor activation (Post et al., 1986, Yaksh and Hammond, 1982).

Both peripheral and CNS pools of 5-HT play an important role in the modulation of nociception (Basbaum and Fields, 1984; Besson and Chaouch, 1987). The involvement of 5-HT in descending control of pain has long been recognized: *p*-chlorophenylalanine (PCPA), which blocks 5-HT synthesis, abolishes central stimulation-induced analgesia, as do lesions of the raphe nuclei made electrolytically or by the selective serotonergic neurotoxin 5,6-dihydroxytryptamine (Basbaum, 1981).

Electrical stimulation of NRM has been shown to increase the release or metabolism of 5-HT in the medullary dorsal horn of the rat spinal cord (Rivot et al., 1982) an effect abolished by PCPA pretreatment. Conversely, noxious stimuli (formalin injection into the forepaw) increase 5-HIAA concentration in both the NRM and medullary dorsal horn although these effects are temporally dissociated: the rise in 5-HIAA in the NRM precedes that in the dorsal horn (Puig et al., 1992). CNS pathways of 5-HT, in particular those projecting to the spinal cord, can mediate antinociception and are involved in the expression of the antinociceptive actions of opioids such as morphine (Basbaum and Fields, 1984; Le Bars, 1988; Sawynock, 1989). Indeed, 5-HT turnover can be modulated by noxious stimuli and a spinal action of 5-HT can both induce behavioral antinociception and inhibit dorsal horn neurons

responsive to noxious stimulation (Besson and Chaouch, 1987;• Le Bars, 1988; Solomon and Gebhart, 1988; Roberts, 1990). Systemic morphine increases 5-HT synthesis in the spinal cord, mainly in the dorsal part (Rivot et al., 1984) and antidepressants have been shown to increase 5-HIAA concentrations and potentiate the action of morphine (Puig, 1993).

At the single cell level, 5-HT is generally thought to be an inhibitory transmitter in the dorsal horn. For instance, stimulation of NRM or ionophoresis of 5-HT generally decreases responses to noxious stimuli (Headley et al., 1978; Zemlan et al., 1994). Such inhibitory effects have been reported to be antagonized by the systemic administration of several putative serotonin antagonists such as lysergic acid diethylamide, methysergide and cinanserin (Engberg et al., 1968; Clineschmidt and Anderson, 1970; Proudfit and Anderson, 1974). However there is some evidence that 5-HT can be excitatory upon small cells (possibly interneurones) in laminae I-III (Todd and Millar, 1982). This is not a non-specific effect since it can be blocked by methysergide (Todd and Millar, 1984).

In contrast with the reasonably clear evidence that α_2-adrenoreceptors mediate the antinociceptive actions of NA, it is far from clear which 5-HT receptors mediate its spinal antinociceptive effects. 5-HT release is at least partly under 5-HT_{1B} control since RU 24969 decreases 5-HIAA concentrations in the medullary dorsal horn (Puig et al., 1993). 5-HT_{1A} receptors are implicated in the inhibitory actions of 5-HT as 50-60% of the wide dynamic range cells in the dorsal horn inhibited by PAG stimulation are also inhibited by iontophoresis of the 5-HT_{1A} agonists 8-OH DPAT (8-hydroxy-2-(di-n-propylamino)tetralin) and buspirone (Zemlan et al., 1994). However, it is possible that some of this effect is indirect, mediated via NA terminals.

At the behavioural level, the data are confusing. One study has found that intrathecal 5-HT_{1A} agonists facilitate the tail-flick reflex (i.e. induce behaviour typical of hyperalgesia) while TFMPP and CGS 12066B (7-trifluoromethyl-4-(4-methyl-1-piperazinyl)-pyrrolo [1,2-a]quinoxaline)(5-HT_{1B} agonists) prolong the tail flick latency (i.e. induce apparent analgesia)(Alhaider and Wilcox, 1993). Another study found that intrathecal 5-HT_{1A} agonists were antinociceptive whereas 5-HT_{1B} agonists were inactive (Mjellem et al., 1992). Antinociception has also been reported after intrathecal 5-HT_3 agonists (Alhaider et al., 1991). Interestingly, in the light of Millar's suggestion (Todd and Millar, 1982) that 5-HT stimulates GABA-ergic interneurones, GABA antagonists blocked this effect. Moreover, activation of spinal 5-HT_2 receptors by intrathecal of 1,(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) has been reported by others to lead to algesic response (hind-limb abduction) that can be blocked by ketanserin (Kjϕsvik et al., 1994).

As well as the projection to the dorsal horn, there is a strong 5-HT projection to the ventral horn, and in this region different receptors may be active. 5-HT depresses the mono- and polysynaptic reflex in neonatal rat spinal cord (Wallis et al., 1993a). 8-OH DPAT preferentially attenuates the monosynaptic reflex (Wallis et al., 1993b) although both responses were blocked by ketanserin suggesting that the effects are mediated through 5-$HT_{2A/2C}$ receptors.

The Neural Pathways Involving in NociceptiveTransmission and Modulation

Between the stimulus of tissue injury and the subjective experience of pain is a series of complex electrical and chemical events. There are four distinct processes involved: transduction, transmission, modulation, and perception.

Figure 4 is a greatly simplified diagram of the pain system. Embedded in the various tissues are nerve endings that respond best to noxious stimuli. *Transduction* is the process by which noxious stimuli lead to electrical activity in the appropriate sensory nerve endings. The second process, *transmission*, refers to the neural events subsequent to transduction. Once the noxious stimulus has been coded by the impulses in the peripheral nerve, the sensations that result are determined by the neurons of the nociceptive transmission system. There are three major neural components of the pain transmission system: the peripheral sensory nerves, which transmit impulses from the site of transduction to their terminals in the spinal cord; a network of relay neurons that ascend from the spinal cord to brain stem and thalamus; and reciprocal connections between thalamus and cortex.

Modulation, the third process, refers to the neural activity leading to control of the pain transmission neurons. A distinct pathway has been discovered in the central nervous system that selectively inhibits pain transmission cells at the level of the spinal cord. This pathway can be activated by stress or by certain analgesic drugs like morphine. When the pain modulation system is active, noxious stimuli produce less activity in the pain transmission pathway.

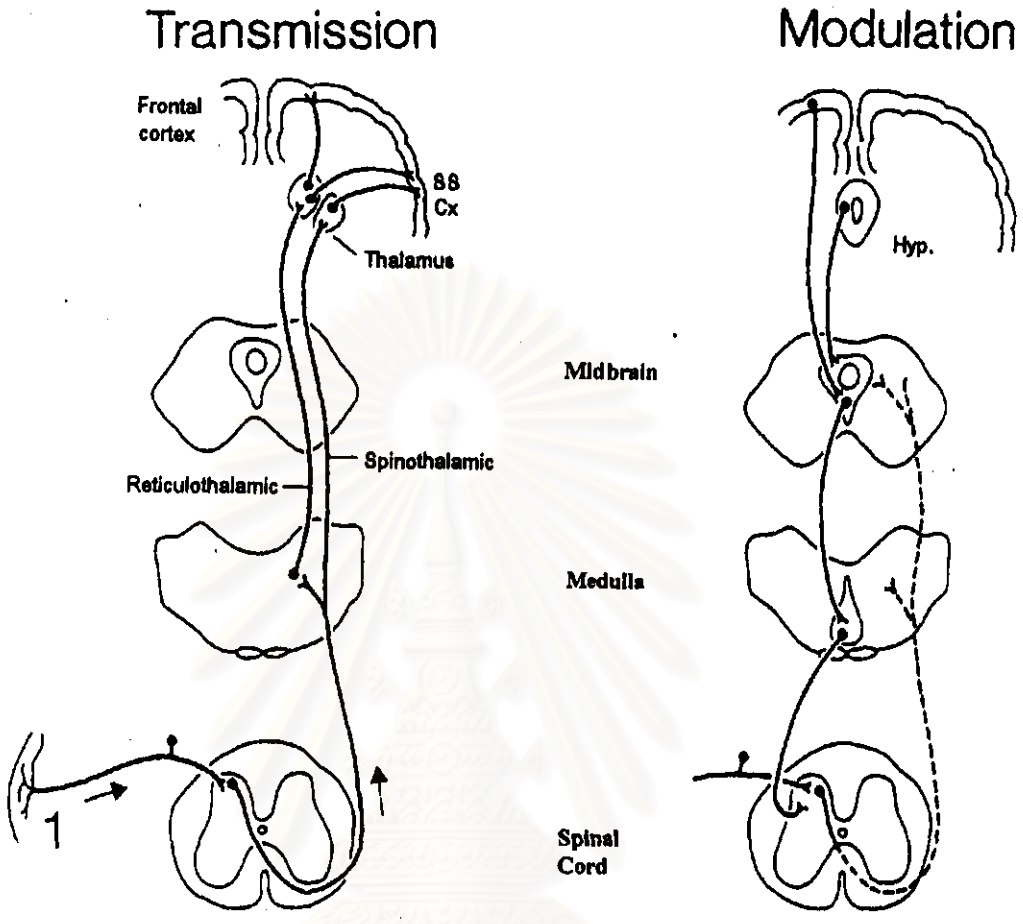

Figure 4. Left: Transmission system for nociceptive messages. Noxious stimuli activate the sensitive peripheral ending of the primary afferent nociceptor by the process of transduction (1). The message is then transmitted over the peripheral nerve to the spinal cord, where it synapses with cells of origin of the two major ascending pain pathways, the spinothalamic and spinoreticulothalamic. The message is relayed in the thalamus to both the frontal cortex and the somatosensory cortex (SS Cx). **Right**: Pain modulation network. Inputs from frontal cortex and hypothalamus (Hyp.) activate cells in the midbrain, which control spinal pain transmission cells via cells in the medulla. (Fields, 1987).

The final "process" is *perception.* Somehow, the neural activity of the pain transmission neurons produces a subjective correlate. How this come about is totally obscure, and it is not even clear in which brain structures the activity occurs that produces the perceptual event.

Pain messages originating from nociceptors are mainly conducted by thin myelinated Aδ- and unmyelinated C-fibers which terminate in the dorsal horn of the spinal cord . When pain signals arrive at this level they cause the release of various transmitters, including excitatory amino acids and neuropeptides such as substance P, neurokinin A, calcitonin gene-related peptide, etc. Some of them activate nociceptive projection neurons, which relay pain signals to thalamic and other nuclei, especially through the spinothalamic tract, which is conceptually two tracts. The direct spinothalamic tract crosses to the contralateral anterolateral white matter of the spinal cord and ascends through the lateral edge of the medulla, lateral pons, and mid brain to the ventrobasal region of the thalamus. From here, thalamic neurons project to the somatosensory cortex. This pathway transmits aspects of acute pain (e.g., location, intensity, quality) and alerts the individual to biologically threatening events. It is also known as the neospinothalamic tract (Fig. 5).

The spinoreticular system mediates autonomic and affective reaction to pain. These ascending fibers terminate in the brain stem reticular formation, pontine, medullary areas, and the medial thalamic nuclei. This tract contributes to affective processing of nociception by connecting ascending information from the brain stem to limbic structures via the noradrenergic bundles (Chapman, 1992).This pathway appeared before the direct spinothalamic projection and thus is also known as the paleospinothalamic tract (Fig.5).

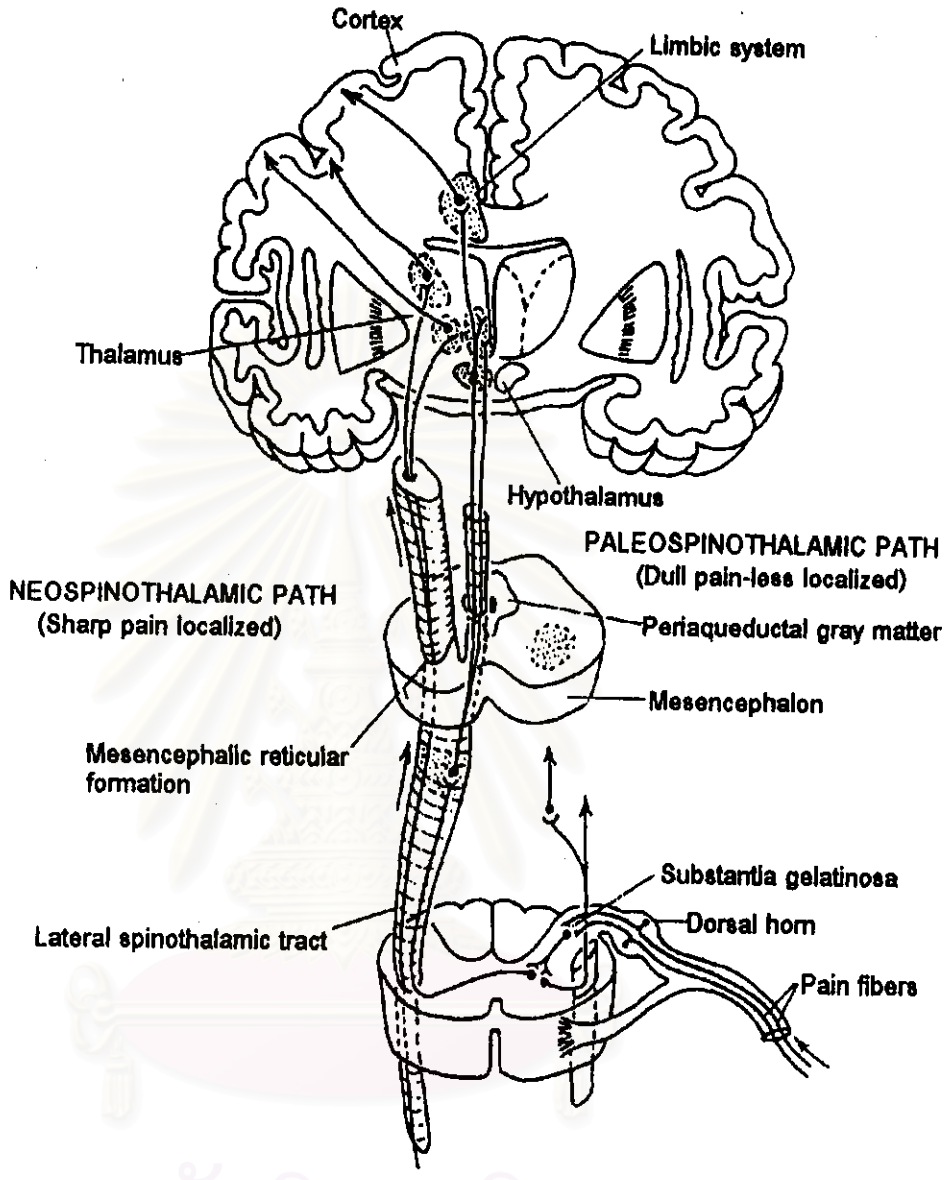

Figure 5. Two major ascending pathways transmit nociceptive information from the dorsal horn of the spinal cord to higher centers : the direct spinothalamic tract (neospinothalamic) and the spinoreticular tract (paleospinothalamic) (Pansky,1992).

Modulation of Pain

Transmission of nociceptive messages at the spinal level is under both segmental and suprasegmental controls (Anderson and Yokata, 1987; Chaouch and Besson, 1986). Melzac and Wall (1965) drew attention to this modulation when postulating their "gate control theory" in 1967. They demonstrated that cells in lamina V of the dorsal horn were more responsive in the decorticated cat (spinal cord blocked) (Wall, 1967), indicating that structures in the brain stem can inhibit or modulate nociceptive input at the level of the spinal cord. In addition, the gate theory postulated that dorsal horn cells could modulate input from the periphery (Wall, 1967).

Supraspinal descending signals also modulate nociceptive input. Stimulation of the periaqueductal grey (PAG) matter causes negative modulation of pain. This interaction was demonstrated experimentally by Reynolds (1969) who showed that painless surgery could be performed on experimental animals if the PAG matter was activated (Mayer, 1971). Additional work by Mayer and Price (1976) demonstrated that higher centers in the nervous system clearly modulated nociceptive input from tissue injury.

Descending Modulating Pathways

Such factors as arousal, attention, and emotional stress can alter response to pain by involving CNS mechanism. A network linking the hypothalamus with the brain stem has been described, which is sensitive to opioids, influences dorsal horn neurons, and triggers their ascending nociceptive transmissions. Hagbarth and Kerr

ascending sensory input. This description was strengthened by the experimental observation of stimulation-produced analgesia (SPA) in animals (Reynolds, 1969; Mayer and Liebeskind, 1974) and in humans with chronic pain (Boivie and Meyerson, 1982; Baskin et al., 1986), during which stimulation of specific brain areas inhibits incoming noxious nociceptive afferents and results in analgesia. Inhibition of dorsal horn cells involved in afferent transmission of nociception to higher centers is accomplished by stimulation of analgesic areas in the brain stem (Guilbaud et al., 1973).

The descending pathway modulating pain has three major components (Fig.6).

1. Neurons in the periventricular and periaqueductal gray matter in the midbrain make excitatory connections in the rostroventral medulla, a region that includes the serotonergic nucleus, raphe magnus and the adjacent nucleus reticularis paragigantocellularis.
2. Neurons in the rostroventral medulla make inhibitory connections in laminae I, II, and V of the dorsal horn; these laminae are also the site of termination of nociceptive afferent neurons. Stimulation of these rostroventral medullary neurons inhibits dorsal horn neurons, including spinothalamic tract neurons that respond to noxious stimulation. Other descending fiber systems that originate in the medulla and pons also terminate in the superficial dorsal horn and suppress activity in nociceptive dorsal horn neurons.
3. Local circuits in the dorsal horn mediate the modulatory actions of the descending pathways. The organization of these local circuits is considered later.

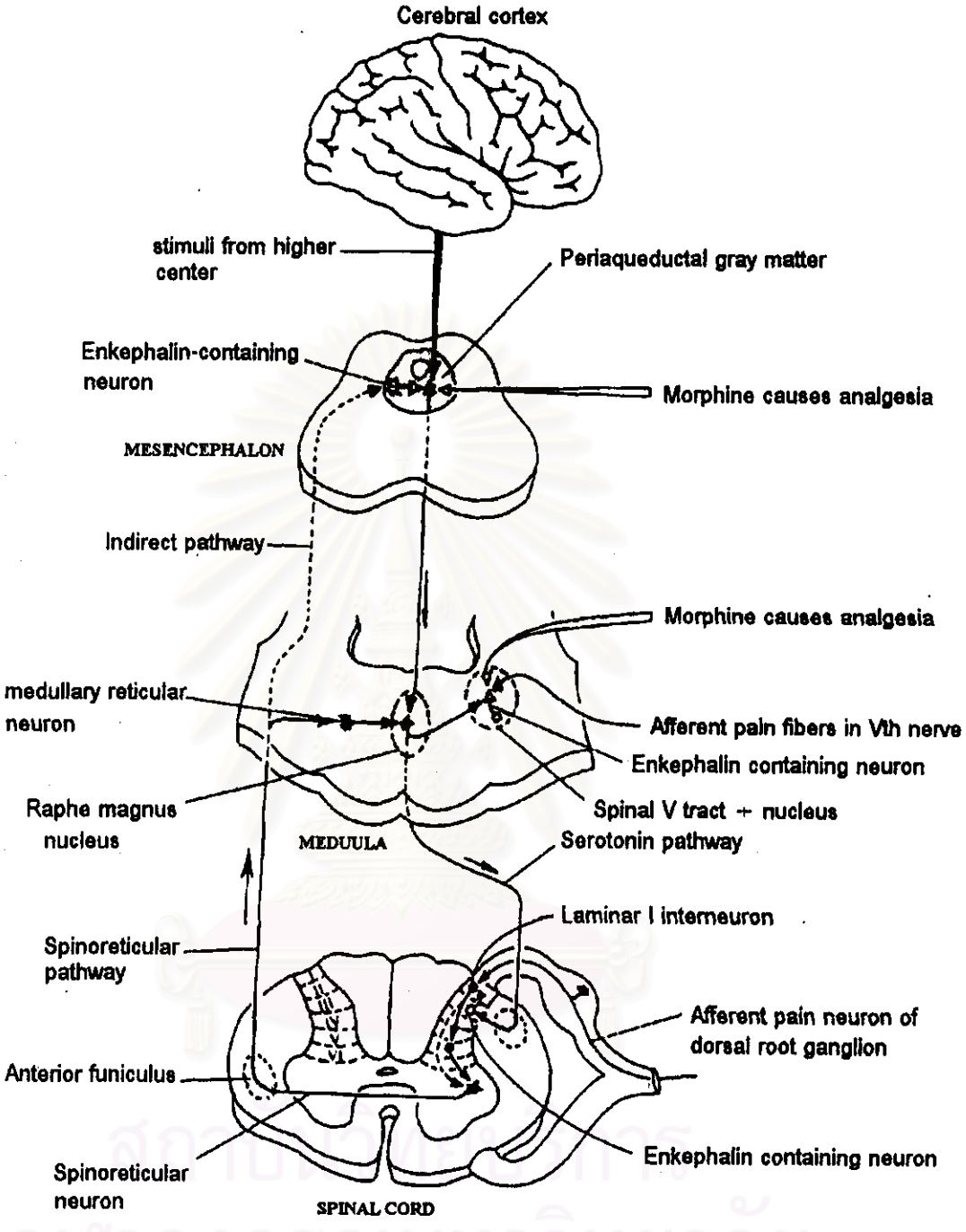

Figure 6. The descending modulating pathway includes connections from the midbrain periaqueductal gray region to the medullary nucleus raphe magnus and other serotonergic nuclei to the dorsal horn of spinal cord. Endorphin-containing interneurons in periaqueductal gray and dorsal horn play an active role in pain modulation. (Pansky B, 1992).

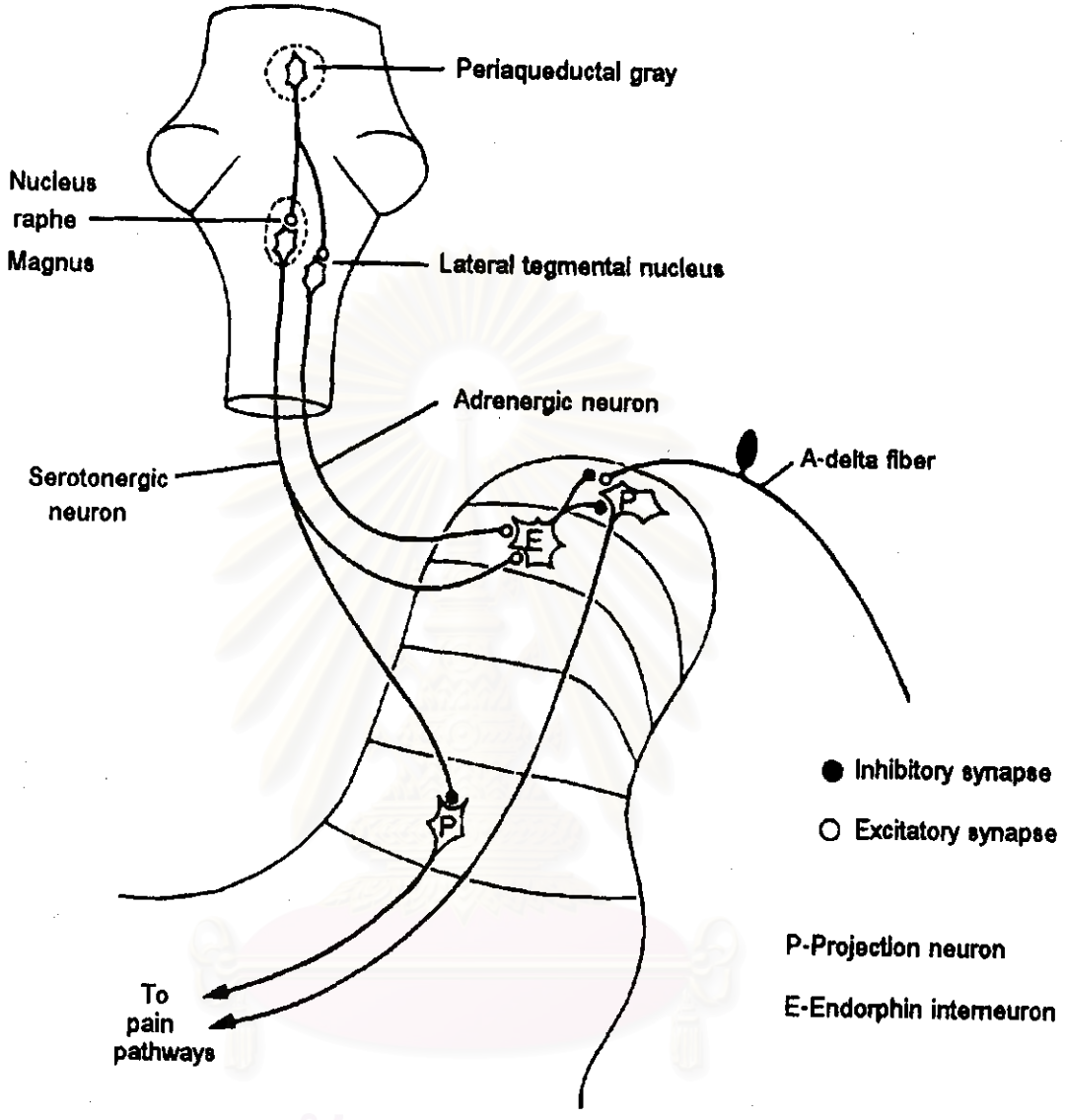

Figure 7. Pain control and modulation. Pain can be modulated by the release of opioid peptides. Neurons of the periaqueductal gray (PAG) matter of the midbrain have excitatory synaptic connections with serotonergic neurons in nucleus raphe magnus and with noradrenergic neurons in the lower brain stem reticular formation. The 5-HT neurons (1) have inhibitory synapses with the nociceptive projection neurons and (2) excitatory synapses with the endorphin-containing interneurons (E) which have inhibitory synapses with nociceptive projection neurons (P). (Charles et al., 1996).

Periaqueductal Gray Matter

Inputs from the frontal cortex (Hardy and Leichnetz, 1981), amygdala (Gray and Magnuson, 1992) and hypothalamus (Reichling and Basbaum, 1990) activate cells in the midbrain (i.e., PAG). Neurons descending from the midbrain synapse in the medulla at the midline nuclei (nucleus raphe magnus) before descending to the dorsal horn. Pathways ascending from the PAG to the medial thalamus and orbital cortex also may control nociception in an ascending portion (Coffield, 1992). Inputs to the PAG also are received from nucleus cuneiformis, the locus coeruleus (origin of the descending adrenergic bundle), and other brainstem catecholaminergic nuclei (Herbert and Saper, 1992). The PAG contains large quantities of all the endogenous opioid peptides. The rostral ventromedial medulla (RVM) includes the midline nucleus raphe magnus, the adjacent reticular formation, and the nucleus reticularis gigantocellularis. Input is received from the PAG and nucleus cuneiformis. Stimulation of opioid receptor in the PAG may influence nociception by altering the descending modulating pathway.

The RVM contains opioid receptors as well as receives neurons from the raphe nucleus that utilize 5-HT as a neurotransmitter (Bitz, 1982). Opioid inhibition of pain at the level of the brainstem and PAG can be reversed by coadministering 5-HT and norepinephrine antagonists at the level of the spinal cord (Yaksh, 1979). 5-HT and norepinephrine neurons are the major descending modulators to the dorsal horn and function to inhibit and modulate nociception. Norepinephrine works through the α_2-adrenergic receptor (Yeomans et al., 1992) and 5-HT via the 5-HT_2 receptor at the spinal level (Crisp et al., 1991).

Axons from the RVM descend to the spinal cord via the dorsal lateral funiculus (DLF), which terminates primarily in the superficial region of the dorsal horn as well as in lamina V. RVM stimulation inhibits dorsal horn ascending nociceptive transmission; this effect can be blocked by lesions in the DLF. Lesions or local anesthetic injections into the RVM abolish the analgesia produced by stimulation of the PAG (Bitz, 1982); opioids injected into the PAG produce analgesia and activate pain-inhibiting neurons in RVM (Cheng et al., 1986). This finding provided evidence that descending modulation from PAG is relayed through the RVM.

Serotonin Transporters

The uptake of 5-HT into cells is mediated by specific sodium-ion coupled transport systems or transporters which function to reduce extracellular levels of 5-HT. These transporters are located on presynaptic nerve terminals (Figure 8) where they act as neurotransmitter inactivation mechanisms to reduce synaptic cleft concentrations of released 5-HT (Iversen, 1971; Snyder, 1970). The presence of 5-HT transporters also permits other cell-types such as platelets and mast cells to concentrate and store 5-HT subsequent secretions (Sneddon, 1973; Kenigsberg and Trifaro, 1980). These active transport systems play an important role in 5-HT homeostasis. A number of drugs which inhibit sodium-ion coupled 5-HT transport produced pronounced psychopharmacological effects. On this basis the 5-HT transporters in the CNS are now considered to be the primary target sites of action for some antidepressants (Graham and Langer, 1988; Shopsin et al., 1981). The physiological, pharmacological and clinical importance of the 5-HT transporters has therefore prompted efforts to characterize more fully these macromolecular entites. This objective has been achieved through the development of selective 5-HT uptake inhibitors which have been subsequently utilized in radiolabelled forms as probes to specifically label the transporters.

Mechanism of serotonergic reuptake

The driving force behind the transport is a chemiosmotic ion gradient over the cell membrane. These gradients are established by the electrogenic Na^+/K^+-ATPase. Serotonin transport is considered to be a two-step process. The first step involves co-transport of 5-HT with Na^+ and Cl^- (K_{in}) into the cell. The second step involves K^+ (or

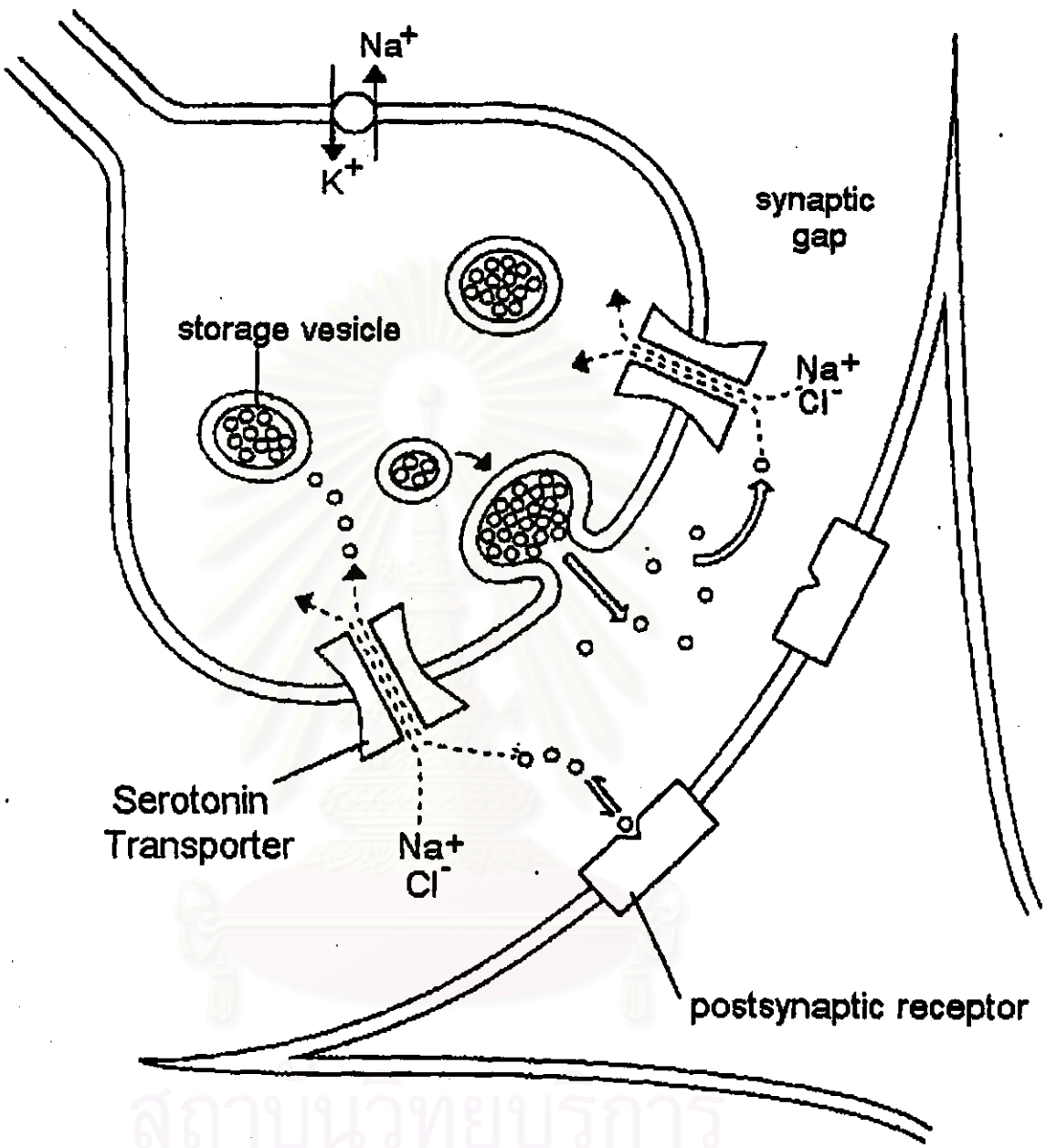

Figure 8. A schematic model of a serotonin synapse. Upon the depolarization induced exocytotic release of 5-HT the sodium-ion coupled transporter located on the presynaptic terminal functions to reduce synaptic gap concentrations of the 5-HT (Graham D and Solomon ZL, 1992).

H^+) translocation from the cell interior to the medium (K_{out}). K_{out} appears to be rate-limiting. 5-HT reuptake can be suppressed specifically by reuptake inhibitors like imipramine. These compounds bind to the transporter but are not translocated over the cell membrane.

Biochemical Studies of 5-HT transporter

The availability of radiolabelled forms of 5-HT uptake inhibitors has provided probes to label the 5-HT transporter in different cell types and tissues. In initial studies [^{3}H]imipramine was used as a marker of both cerebral cortical and platelet (Langer et al., 1980) forms of the 5-HT transporter. However, with the development of selective and potent inhibitors of sodium-ion coupled 5-HT uptake including fluoxetine (Stark et al., 1985), sertraline (Reimherr et al., 1988), citalopram (Hyttel, 1982), and paroxetine (Thomas et al., 1987). [^{3}H]imipramine has to a large extent been superceeded by radiolabelled forms of these selective uptake blockers, particularly [^{3}H]paroxetine (Segonzac et al., 1987; Habert et al., 1985) and [^{3}H]citalopram (D'Amato et al., 1987). The specificity of radiolabeled 5-HT uptake inhibitors such as [^{3}H]paroxetine as selective markers of the 5-HT transporter has, moreover, been exploited for mapping serotonergic pathways in rat and human brain using in vitro and in vivo autoradiographic studies (De Souza and Kuyatt, 1987; Cortes et al., 1988; Scheffel and Hartig, 1989).

Imipramine binding site

Imipramine binding was first measured in platelet plasma membranes (Rudnick and Talvenheimo, 1978; Talvenheimo et al., 1979) and subsequently at sites

in brain and intact platelets (Raisman et al., 1979; Paul et al., 1980) which have been nominated as receptors for endogenous "mood control" hormones (Briley et al., 1979). In isolated platelet plasma membrane vesicles, imipramine binds to a single class of sites with characteristics identical with those of the Na^+-dependent transporter responsible for serotonin accumulation by platelets and nerve endings.

The imipramine binding site (IBS) is presumed to be identical to a functionally associated with the actual 5-HT carrier. Three observations led to this assumption. Firstly, the regional distribution of the IBS closely corresponds to that of 5-HT innervation (Kovachich et al., 1988; Langer et al., 1981). Secondly, Sette et al. (1981 and 1983) demonstrated that denervation of serotonergic neurons in the dorsal raphe nuclei using 5,7-dihydroxytryptamine or electrolytic lesions eliminated both the IBS and the 5-HT transporter to the same extent. Thirdly, a significant correlation between the displacement profile of [^{3}H]imipramine binding and inhibition profile of 5-HT reuptake has been reported by various research groups (Langer et al., 1980; Segonzac et al., 1987). Although the functional association of the IBS with 5-HT reuptake is commonly accepted, the nature of the coupling between identity of the IBS and the 5-HT-carrier is still subject to considerable uncertainty. Several lines of evidence support the hypothesis that the system consists of a mutually exclusive regulatory and carrier site. In 1983, Barbaccia et al. showed that down-regulation of the IBS density after chronic administration of imipramine was accompanied by an up-regulation of 5-HT reuptake in rat hippocampus (Barbaccia et al., 1983). Differences in the Na^+ requirement of imipramine binding and 5-HT transport were reported (Talvenheimo et al., 1983).

Imipramine binding is a Na^+-dependent and competitive with 5-HT, as expected if binding occurs at the normal substrate-binding site of the transporter. The lack of detectable imipramine transport (Talvenheimo et al., 1979) suggests that imipramine binding could be used as a probe of the steps leading to formation of the transporter complex with 5-HT, Na^+ and Cl^-. Previous studies using porcine platelet plasma membrane vesicles demonstrated a requirement for more than one Na^+ ion for maximal imipramine binding, although a single Na^+ ion is apparently co-transported with 5-HT (Talvenheimo et al., 1983).

High-affinity [^{3}H]imipramine binding sites in brain

High-affinity specific binding of [^{3}H]imipramine was first demonstrated in the rat brain (Raisman, 1980) and has subsequently been found in the brain of several animal species including man (Langer, 1980; Rehavi et al., 1980). The properties of [^{3}H]imipramine binding were summarized in Table 2 and compared to the generally accepted criteria for the identification of a binding site as the specific site of drug action or of a pharmacological receptor.

[^{3}H]imipramine binds to a smaller number of high affinity sites in the brain (K_d, 4 nM; maximal binding, B_{max} 16 pmol/g tissue in the rat hypothalamus.). The specific binding of [^{3}H]imipramine is rapid and reversible, the addition of 10 μM desipramine displacing the specific binding completely with a half time of 5 min.

The specific binding of [^{3}H]imipramine is unevenly distributed in the rat brain, the highest density of binding sites being found in the hypothalamus and the lowest density in the cerebellum.

Table 2. Criteria for the binding of a radioligand to a specific site of drug action (or a receptor) and the properties of specific [^{3}H]imipramine binding

Criteria	Properties of [^{3}H]imipramine binding
Binding parameters	
(1) High-affinity	K_d = 4 nM
(2) Saturable	Yes-Hill coefficient = 0.97
(3) Limited No. of sites	B_{max}(hypothalamus) = 16 pmol/g tissue
(4) Rapid kinetics	T1/2 = 5 min at 0°C and 3.5 nM
(5) Affinity from kinetic constants and equilibrium constants should be the same	K_d (kinetic) = 6.8 nM K_d (equilibrium) = 4.0 nM
Distribution	
(6) Asymmetrical regional distribution	Five fold difference between the richest and poorest brain region
(7) Asymmetrical tissue distribution	Found only in brain and platelets
(8) Cellular distribution	Not found in glial cells
Selectivity	
(9) Pharmacological selectivity	Only tricyclic antidepressants and 5-HT uptake blockers inhibit the binding with high affinity
(10) Stereoselectivity	Z-forms of Zimilidine and 10-OH tricyclic antidepressant inhibit with much greater affinity than E-forms
(11) Sensitivity to ions	Sensitive to Na^+ ions
Functional correlations	
(12) Correlation with pharmacological effects	Positive correlation with the inhibition of 5-HT uptake
(13) Correlation wih clinical effects	Positive correlation with mean clinical doses of tricyclic antidepressants
(14) Correlation with pathological conditions	[^{3}H]imipramine binding decreased in the platelets of untreated depressed patients

Platelet and Serotonin

Although blood platelets lack the general characteristics of either 5-HT neurones or neurones with postsynaptic 5-HT receptors and are unable to synthesize 5-HT, they are able to take up, store and release 5-HT via mechanisms that are sufficiently similar to those of 5-HT neurones to render the platelets interesting as a model for the 5-HT neurone (Pletscher, 1968; Sneddon, 1973).

Platelets and serotonergic nerve ending share many morphological, biochemical and pharmacological characteristics (Malmgren and Hasselmark, 1988; Campbell et al., 1981; Pletscher and Laubscher, 1980; Peters and Grahame-Smith, 1980; Langer et al., 1981).Their respective cell membranes contain similar supplies of receptors ($alpha_2$, $beta_2$, 5-HT_2) and specific imipramine binding sites (Langer et al., 1981). Both cell types also have lipid metabolizing systems and the intracellular mediates of secretion invole Ca^{2+} and metabolites of the phosphatidylinositol, phosphate cycle and the prostanoid pathway, enzymes which are common in both cells, such as monoamine oxidase (MAO), gamma aminobutyric acid (GABA) aminotransferase and neuron specific enolase (NSE). Moreover, a common origin of platelets and serotonergic neuron from the embryonic has been suggested (Campbell at al., 1981).

Platelets have also proven to be a reliable and predictive model for the pharmacological and biochemical characterization of the 5-HT-reuptake system. The 5-HT-reuptake system in platelets and neurones share many similarities. Both systems possess two separate carriers for serotonin: rather non-specific ATP-dependent system, which is reserpine-sensitive, and a specific, imipramine-sensitive, Na^+-

coupled reuptake system. The pharmacological profile of the latter in platelets strongly correlated with that in brain synaptosomes (Da Prada et al., 1982). Additionally, kinetics of transport of both systems are similar. 5-HT reuptake in platelets, on the other hand, appears to be less selective to 5-HT, in that it is also capable of transporting noradrenaline, dopamine and tryptamine (Malmgren, 1986; Segonzac, 1985). The IBS-receptor distribution in the brain of Fawn-Hooded rats with a hereditary deficiency in platelet 5-HT storage is not markedly changed, implying a different type of genetic control (Ieni et al., 1985). In summary, decisive evidence on the identity of both systems is lacking, but the similarities suggest that the platelet is an appropriate model system for studying neuronal 5-HT reuptake.

Moreover, the metabolism of 5-HT in the platelet dense body is said to mirror metabolism in the nervous system (Malmgren and Hasselmark, 1988). Almost all of the 5-HT present in blood is located in platelet dense bodies and is released upon platelet activation (Hardisty and Stacey, 1955). Alterations in platelet 5-HT uptake and MAO activity have been reported by many reseachers in several psychiatric and neurological disorders. Reduced imipramine binding has been found on the platelets of depressed patients (Briley et al.,1980) as well as in the frontal cortex of suicide victims (Stanley et al., 1982) and depressed patients dying from natural causes (Perry et al., 1983). These findings suggested the potential value of the platelets as an indicator of changes in central serotonergic function.

The above and other findings led to the conclusion that platelets can be used as models for studying the central 5-HT neurons. Figure 9 summarizes some of the similarities between platelets and 5-HT neurons.

Platelets as a model for the 5-HT Neuron

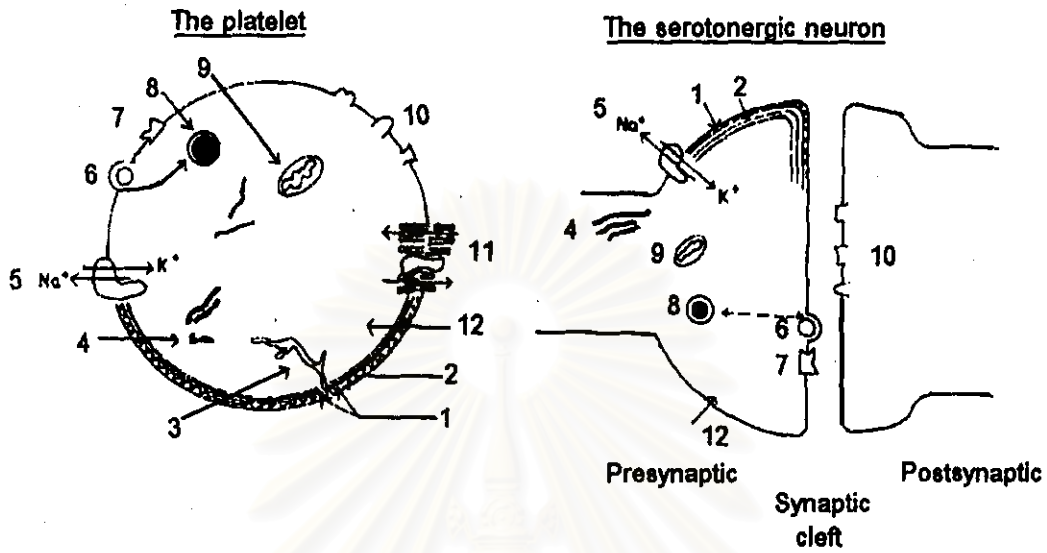

Figure 9. The structural similarities between platelets and serotonergic neuron

1. Contractile microfilament
2. Microtubules
3. Surface connecting system
4. Dense tubules
5. Quabain sensitive Na^+/K^+-ATPase
6. 5-HT uptake system
7. Imipramine binding site
8. Dense body: storage of 5-HT, Ca^{2+}ATP, ADP and enkephalin
9. Mitochondrion with membrane bound MAO
10. α_2- and β_2-adrenoceptors, 5-HT_2-receptors
11. Passive diffusion of small molecules through phospholipid bilayer
12. Neuron-specific enolase (NSE) and γ-aminobutyric acid (GABA) amino-transaminase

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

The nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of nonopioid analgesics. It is generally assumed that the analgesic effect of NSAIDs is due to inhibition of PG synthesis in the periphery. For the majority of these agents, this is considered to be their principal mode of action in the relief of pain secondary to chronic inflammation.

Basically, NSAIDs suppress the formation of prostaglandins from arachidonic acid, and thus remove the sensitizing action which these lipids have on nociceptors (Figure 2).

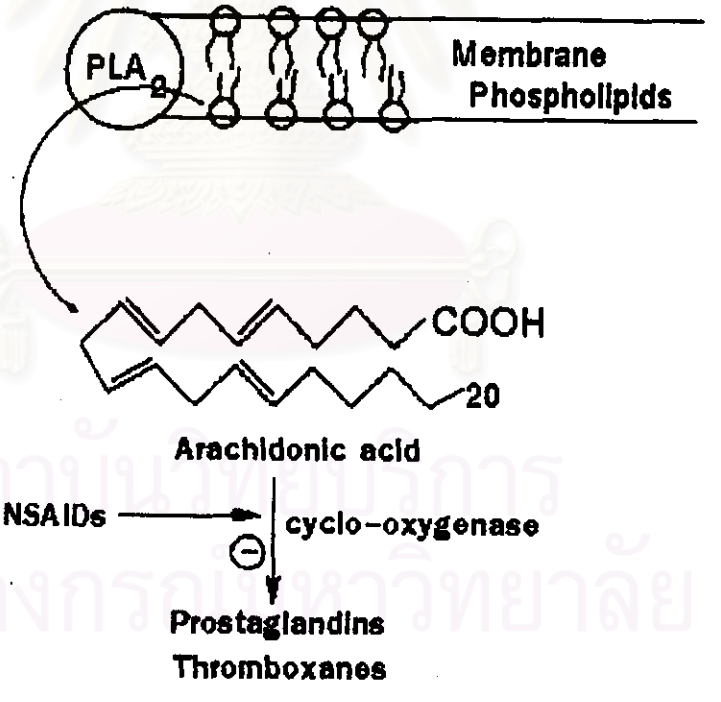

Figure 10. NSAIDs act by preventing the conversion of arachidonic acid into prostaglandins and thromboxanes. Arachidonic acid is split off from membrane lipids by phospholipase A_2 (PLA_2). (Craig CR and Stitzel RE, 1997).

The physiological role proposed above for PGs is consistent with the hypothesis that the analgesic action of NSAIDs results from their effect on local PG synthesis. However, there is now abundant evidence to suggest that inhibition of locally synthesized PGs *per se* does not satisfactorily explain the analgesic effect of these agents in many models of clinical pain (Bannwarth et al., 1993; Biella and Groppetti, 1993; Brune et al., 1993; Capetola et al., 1983; McCormack and Brune, 1991; Weissman, 1992; Dupont et al., 1993), and that for many of these compounds a central action must be considered.

Central Antinociceptive Modulation by NSAIDs

The traditional belief that the effects of NSAIDs are mediated exclusively through a peripheral mechanism related to inhibition of prostaglandin synthesis has been challenged (Bjorkman, 1995; McCormack 1994; McCormack and Brune, 1991). There is increasing evidence that NSAIDs have a central mechanism of action that augments the peripheral mechanism.

In Vivo Evidence for Central Antinociception of NSAIDs

Evidence from animal studies that NSAIDs may elicit some of their analgesic effect within the CNS is based on behavioural response to injection of algogenic substances and measurement of evoked activity within the CNS in response to electrical stimulation. The behavioral response to intraperitoneal injection of formalin and acetic acid is biphasic (Hunskaar, 1987). The short latency of the immediate response reflects a central mechanism of action, while the delayed hyperalgesia represents pain secondary to inflammation. In this animal model, aspirin and

morphine have been shown to have an almost identical time-effect relationship on the immediate response (Hunskaar, 1987). Carlsson et al.(1988) found that the rat thalamic response to electrical stimulation of peripheral nociceptive afferents was depressed in a dose-dependent manner by aspirin and paracetamol, and that this effect was not reversed by naloxone. Using the same rat model, Jurna and Brune (1990) found that indomethacin, ibuprofen and diclofenac produced a dose dependent depression of evoked activity, suggesting a central analgesic effect. Furthermore, NSAIDs prevent the rise in cerebrospinal fluid prostaglandins after activation of the NMDA receptor (Bjorkman, 1995). Spinally administered ibuprofen has an antinociceptive effect, which is stereoselective (Wang and Hiller, 1995).

Clinical Evidence for Central Antinociception

Evidence from studies in human volunteers and electrophysiological studies in paraplegic patients shows that NSAIDs may elicit some of their analgesic effect within the CNS (Bjorkman, 1995; McCormack, 1991, 1994). Howver, the usefulness of somatosensory evoked potentials in the assessment of analgesic efficacy has been questioned (Moore et al., 1995). It has also been noted that intrathecal lysine acetylsalicylate can relieve intractable pain in humans (Devoghel, 1983).

Proposed Mechanism for Central Antinociception of NSAIDs

Various mechanisms have been suggested to account for the central action of NSAIDs. A central antinociceptive effect may be the result of interference with the formation of prostaglandins or with transmitter or modulators in the nociceptive system. Alternatively, the central action may be mediated in part by endogenous

opioid peptides or blockade of the release of serotonin or even by a mechanism mediated by inhibition of excitatory amino acids or NMDA receptors (Jurna and Brune, 1990).

1. Central Prostaglandin Synthesis

An inhibitory effect on central prostaglandin synthesis is supported by the finding that a number of NSAIDs reduce in vivo formation of E and F-prostaglandins in the CNS; diclofenac, indomethacin and naproxen have displayed dose-dependent inhibition (Abdel-Halim, 1978). In addition, diclofenac may reduce prostacyclin formation in the thalamus (Attal et al., 1988).

2. Opioid Mechanisms

Several studies in amimals and humans have applicated a central opioid mechanism of action of NSAID-mediated antinociception (Bjorkman, 1995; Martini et al., 1984; Saccerdote et al., 1985; Vescovi et al., 1987; Groppetti et al., 1988). It has been shown in animal models that antinociceptive effects of diclofenac (Bjorkman, 1995) and ketorolac (Domer, 1990) can be reversed by naloxone. Bjorkman, 1995 was unable to determine whether the interaction of diclofenac with opioid mechanisms was direct or indirect in nature; however, both lysine acetylsalicylate and diclofenac have been reported to reduce the heroin withdrawal syndrome in humans, which would been to implicate a direct pharmacological interaction (Gerra, 1983, 1985).

3. Serotonergic Mechanisms

There is abundant evidence to support a direct relationship between central serotonergic mechanisms and the antinociceptive effect of NSAIDs (Bjorkman, 1995;

McCormack, 1994). A correlation between analgesia induced by sodium salicylate and the increase in the turnover rate of dopamine, noradrenaline and 5-HT in the brain stem of the rat has been proposed by Bensemanna and Gascon (1978). Shyu and Lin (1985) observed that the analgesia induced by intrahypothalamic administration of acetylsalicylic acid (ASA) was antagonised by the pretreatment of monkeys with either the 5-HT receptor antagonist cyproheptadine, or the catecholaminergic receptor antagonists haloperidol or α_2 antagonist. Gropetti et al. (1988) demonstrated in rats that intravenous administration of ASA reduced the firing discharge of thalamic neurons, evoked by noxious stimuli. Concomitantly, concentrations of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) increased, while those of the Met-enkephalin-like immunoreactives were decreased in several areas of the brain. The depressive effect of ASA on the evoked neuronal firing was counteracted by pretreatment with the 5-HT receptor antagonist metergoline, but not by the opioid receptor antagonist naloxone. Moreover, the administration of either of the 5-HT precursor compounds, tryptophan or 5-HTP also inhibited the neuronal activity evoked by noxious stimulation in addition to increasing the turnover of 5-HT in the brain. These observations are consistent with the view that a 5-HT but not a naloxone-sensitive opioid mechanism, may be relevant for acetylsalicylate/ salicylate-mediated analgesia (Longheu et al., 1988).

The activation of the descending serotonergic inhibitory pathway has been proposed as a mechanism to account for the analgesic effect of some non-acidic NSAIDs, including paracetamol (Tjolsen et al., 1991). Vitale et al. (1992) observed that phenazone 60 mg/kg/day administered orally to adult rats for 30 days increased the maximum number of [^{3}H]-5-HT binding sites in cortical and pontine membranes,

without any effect on binding affinity. In a more recent study, the same group (Sandrini et al., 1993) investigated the acute effects of phenazone on brain 5-HT binding sites. In contrast to chronic treatment, phenazone 60 mg/kg administered orally to adult rats provoked a significant decreased in [^{3}H]-5-HT binding sites both in the pons and cerebral cortex after 2, 4 and 8 hr, but not after 24 hr. Using the hot plate test, phenazone produced a significant elevation in response latency at 2, 4 and 8 hr after administration. The antinociceptive effect was not detectable after 24 hr nor after 15 and 30 days of chronic treatment. Interestingly, although serum levels of phenazone correlated significantly with the time course of this antinociceptive effect, even without correcting for the protein-bound fraction, these levels must be considered too low to inhibit PG biosynthesis (Brune and Alpermann, 1983; Brune, 1990). Accordingly, Sandrini and coworkers (1993) concluded that their results are consistent with the hypothesis that, in part, the analgesic effect of phenazone may be attributable to a drug-induced release of central 5-HT.

In addition, it was founded that diclofenac reduced both brain stem and spinal cord 5-HT and 5-HIAA levels. In animal studies, pretreatment with the tryptophan hydroxylase inhibitor para-chlorophenylalanine (PCPA) potently antagonised the antinociceptive effects of diclofenac (Bjorkman, 1995). It was suggested that diclofenac activates descending serotonin pathways to elicit antinociception, and that this may preferentially involve 5-HT_2 receptor-mediated rather than 5-HT_1 receptor-mediated mechanisms.

4. NMDA or Excitatory Amino Acid Mechanisms

In the rat model, exposure to noxious stimuli results in elevated levels of the excitatory amino acids glutamate and aspartate in spinal cord microdialysates (Smullin et al., 1990). Malmberg and colleagues (1992) have demonstrated in an animal model that spinally administered aspirin, S(+)-ibuprofen and ketorolac all antagonise the hyperalgesia induced by activation of spinal glutamate or substance P receptors. Pretreatment with diclofenac and S(+)-ibuprofen has been shown to reduce hyperalgesia induced by NMDA activation, while the pharmacologically inactive isomer R(-)-ibuprofen had no effect (Bjorkman, 1995). This stereoselectivity strongly suggests a receptor-mediated effect.

It has also been suggested that the antinociceptive effect of NSAIDs such as diclofenac and ibuprofen may in part be attributed to interference with endogenous nitric oxide modulating nociceptive activity at the spinal level (Bjorkman, 1995). Spinal NMDA receptor activation results in enhanced biosynthesis of nitric oxide from arginine. In support of this, pretreatment with the natural substrate for nitric oxide, *l*-arginine, reverses the antinociceptive effect of diclofenac. This action is stereoselective, being unaffected by d-arginine. In contrast, Hunskaar and colleagues (1985), although able to demonstrate an effect of these drugs on excitatory amino acid- or substance P-mediated hyperalgesia.

PHARMACOLOGY OF PARACETAMOL

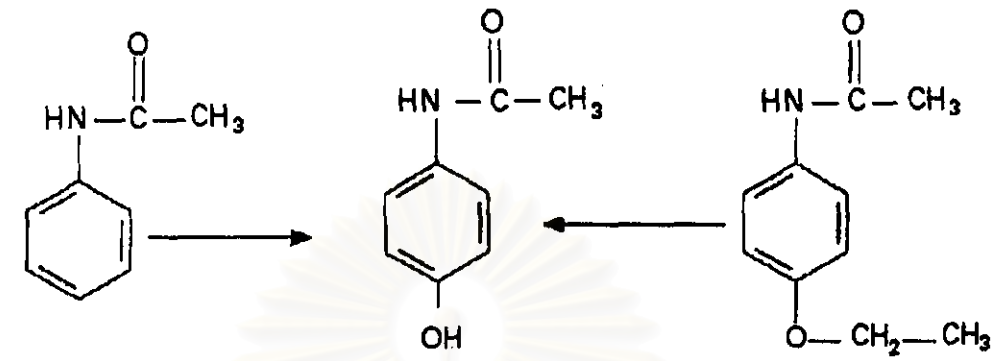

Figure 11. Chemical structures of paracetamol, including *p*-aminophenol derivatives

Paracetamol (Acetaminophen, N-acetyl-para-aminophenol) is the metabolite of two previously used drugs, phenacetin and acetanilid which have the chemical structures as shown in Figure 11. Paracetamol possesses analgesic and antipyretic effects which resemble those of aspirin, but is devoid of anti-inflammatory activity. However, it is usually classified with the NSAIDs as it possesses a similar profile of activity in relief non-specific pain. Alone or in combination with other drugs, it is found in more than 200 formulations promoted for symptomatic relief of pain, cough and colds (Weiss, 1973).

Like aspirin, paracetamol is widely used in the treatment of pain of moderate intensity such as headache, toothache, dysmenorrhea and pains of musculo-skeletal origins. Its popular use is partly due to the low incidence of adverse effects relative to aspirin (Miller, 1976). At therapeutic doses, adverse effects rarely occur with paracetamol. Because paracetamol is widely available and forcefully promoted as a

"safe" aspirin substitute, there is a need to reevaluate its status as an analgesic-antipyretic agent in clinical medicine.

Paracetamol was synthesized at Johns Hopkins University in 1877 and was first used in clinical medicine in 1893 by von Mehring. Its use did not become extensive until 1949, when Brodie and Axelrod recognized it as the principal active metabolite of acetanilid and phenacetin.

Pharmacodynamic Properties of Paracetamol

1. Mechanism of action

The basic pharmacological mechanisms of action of paracetamol has not received the scientific attention accorded to the salicylates and, consequently, many explanations for its activity appear somewhat speculative. It has analgesic and antipyretic properties which do not differ significantly from those of aspirin. However, paracetamol lacks the potent anti-inflammatory actions of aspirin.

Paracetamol, an effective analgesic but only a weak anti-inflammatory agent has not been satisfactorily established, although most recent explanations involve a selective inhibition of some facet of prostaglandin biosynthesis (Flower et al., 1980; Jackson et al., 1984; Meredith and Goulding, 1980; Ramwell, 1981). Some evidence suggests that paracetamol has a weak inhitory influence on peripheral prostaglandin biosynthesis (which would account for its lack of substantial anti-inflammatory activity), but that it is a potent inhibitor of prostaglandin production within the central nervous system (presumably accounting for its analgesic and antipyretic properties).

2. Analgesic Effects

Like the salicylates, paracetamol possesses analgesic activity which is effective against pain of mild to moderate severity. However, unlike the salicylates, which act mainly peripherally against pain associated with inflammation, paracetamol has few or no anti-inflammatory properties and apparently exert its analgesic effects via central actions (Bowman and Rand, 1980; Flower et al., 1980; Jackson et al., 1984). This is supported by the work of Ferreira et al. (1978) which suggests that release of prostaglandins within the central nervous system, involving pain circuits, as well as sensitization of peripheral pain receptors by locally released prostaglandins both contribute to inflammatory hyperalgesia. However, Guzman and Lim (1976) demonstrated that paracetamol relieved pain by blocking impulse generation at bradykinin sensitive chemoreceptors which evoke pain-a peripheral mechanism.

Central serotonergic systems may be involved in paracetamol-mediated antinociception (Pelissier et al., 1994; Tjϕlsen et al., 1991). The analgesic activity of paracetamol was altered in rats in which the serotonergic pathways were lesioned with intrathecal 5,6-dihydroxytryptamine (Tjϕlsen et al., 1991).

It would be hazardous to infer from these observations that paracetamol interact directly with 5-HT receptors in the CNS. The following data should be considered. Firstly, 5-HT-related antinociception is mediated by a 5-HT-induced release of noradrenaline in the spinal cord (Sawynock and Reid, 1992). Accordingly, *in vitro* studies failed to show any binding of paracetamol to 5-HT_3 receptors or any inhibitory effect of this drug on 5-HT reuptake (Pelissier et al., 1994). Secondly, mutual interactions between 5-HT or catecholamine transmitters and opiate neurons

have been reported (Groppetti et al., 1988; Taiwo and Levine, 1988). It is therefore possible that the central antinociceptive properties of paracetamol could be mediated indirectly by opioid mechanisms. Interestingly, naloxone did not abolish the antinociceptive activity effects of ketorolac, aspirin and paracetamol (Carlsson et al., 1988; Groppetti et al., 1988; Uphouse et al., 1993).

Prostaglandin Inhibition and Centrally-Mediated Analgesia of Paracetamol

Despite their common mechanism of action, paracetamol and other NSAIDs differ pharmacologically. At usual doses, floctafenin appears as a simple analgesic whereas paracetamol is devoid of any significant anti-inflammatory effect in humans (Bannwarth et al., 1992). This observation lends clinical support to the assumption that PG independent mechanisms are involved in the properties of NSAIDs, particularly in their anti-inflammatory effects (Abramson and Weissman, 1989). Alternatively, the dissociation of antipyretic, analgesic and anti-inflammatory properties may arise from the heterogeneity in the cyclo-oxygenase enzymes (Flower and Vane, 1974). Thus it is possible that different isoenzymes differ with respect to their susceptibility to drugs (Laburn et al., 1980; Neuman et al., 1987; Weksler et al., 1983). Whether paracetamol selectively impedes cyclo-oxygenase activity in the CNS is however debated (Tolman et al., 1983; Vane, 1987).

Systemically administered paracetamol as well as various NSAIDs were shown to be capable of inhibiting ischemia-induced PGE_2 formation in mouse brain (Ferrari et al., 1990). The degree of inhibition of brain cyclo-oxygenase correlated well with the antinociceptive potencies of these drugs in the acetylcholine-induced constriction test (Ferrari et al., 1990). Nevertheless, non-acetylated salicylates are

generally considered as poor PG biosynthesis inhibitors on the basis of *in vitro* data (Abramson and Weissmann, 1989). Since numerous variables may influence these investigations, their results must be considered cautiously (Neuman et al., 1987; Tolman et al., 1983). Concurrently, significant inhibition of PG production was observed in the brain after analgesic doses of R-flurbiprofen (Brune, et al., 1992). Thus, it was hypothesized that inhibition of PG synthesis within the CNS may account for the central analgesic effect of paracetamol.

Pharmacokinetics of Paracetamol

Paracetamol is a weak acid with a pKa of 9.5. The drug is rapidly absorbed from the gastrointestinal tract, reaching peak plasma levels within 40 to 60 minutes of ingestion (Dordoni et al., 1973). Binding to plasma proteins is variable but considerably less extensive than that of aspirin (Gazzard et al., 1973).

Paracetamol is rapidly and uniformly distributed throughout body tissues; it achieves a tissue: plasma concentration ratio of unity in most tissues except fat and cerebrospinal fluid. At plasma concentrations of less than 60 mg/L. paracetamol does not apparently bind to plasma proteins; at 90 mg/L protein binding was less than 5%; and after toxic doses (plasma paracetamol concentrations of up to 280 mg/L) protein binding varied from 8 to 43% with no correlation between binding and plasma paracetamol concentration (Gazzard et al., 1973).

Only 2 to 5% of a therapeutic dose of paracetamol is excreted unchanged in the urine; the remainder is predominantly metabolized by the liver. At therapeutic dosages, paracetamol is mainly metabolized (>80%) to the glucuronide and sulphate

conjugates. A small amount may be converted into highly reactive metabolites, but these are usually transformed into mercapturic acid compounds by conjugation with glutathione. Large doses, as in cases of poisoning, may overwhelm the available resources for glutathione conjugation. In this event the highly reactive radicals may react with high molecular weight cell constituents, such as proteins or polymeric nucleic compounds, and may thus damage the cells (hepatotoxicity). Paraminophenols and their metabolites are potentially carcinogenic, possibly because of the formation of highly reactive radicals (Nanra, 1980).

To clarify the involvement of 5-HT and analgesic action of paracetamol, we performed a series of experiment to study the integrity of 5-HT system in rats treated with paracetamol acutely for 90 min and chronically for 15 and 30 days. The studied parameters are characteristics of 5-HT_{2A} receptors, 5-HT uptake sites on frontal cortex and brain stem membranes, including the evaluation of antinociceptive activity and platelet 5-HT and 5-HIAA concentrations.

The Purpose of the Study

Evidence from a variety of sources indicated that a central serotonergic system may be involved in analgesic action of paracetamol. In order to clarify this hypothesis, we performed a series of experiment to study the acute and chronic effect of paracetamol in the rats. The aim of this study are:-

1. To study the chronic effect of paracetamol on the binding characteristics of central 5-HT_{2A} receptors in rat brain.
2. To study the chronic effect of paracetamol on the binding characteristics of 5-HT uptake sites in rat brain.
3. To study the chronic effect of paracetamol on the levels of 5-HT and its metabolite 5-HIAA in platelets of rats.
4. To compare the binding characteristics of central 5-HT_{2A} receptors in rat brain between 15 and 30-day paracetamol-treated groups.
5. To compare the binding characteristics of 5-HT uptake sites in rat brain between 15 and 30-day paracetamol-treated groups.
6. To compare the levels of 5-HT and 5-HIAA in platelets between 15 and 30-day paracetamol treated-groups.
7. To study the antinociceptive effect of paracetamol in rats after acute and chronic paracetamol treatment.
8. To compare the rat body weight between 15 and 30-day paracetamol treated group.