

Appendix A14.9

The Serotonergic System

Serotonin (5-HT) was given its name by Rapport et al., (1947) because of its activity as an endogenous vasoconstrictor in blood serum. This was later acknowledged as being the same molecule (secretine) found in the intestinal mucosa and “secreted” by chromaffin cells (Brodie, 1900; Trifaro et al., 1984). Following this, 5-HT soon became characterized as being a neurotransmitter in the CNS (Bogdansky et al., 1956). 5-HT is found in platelets (8%), chromaffin cells of the intestine (90%), and in neurons (2%). The 5-HT system is linked to affective state. 5-HT neurons project to many similar structures as that of the NA and likely reflect their similarities in the regulation of behaviors. The precursor to 5-HT is L-tryptophan, an amino acid that is derived primarily from the diet and crosses the blood brain barrier through a non-specific carrier. However, due to competition with other amino acids for this carrier, only 4% of the circulating tryptophan contributes to 5-HT synthesis in the CNS. Tryptophan depletion via dietary restrictions has been used as a means to assess the role of the 5-HT system in many psychiatric illnesses (Young, 1993).

Synthesis of 5-HT consists of hydroxylation of tryptophan by the enzyme tryptophan hydroxylase in the presence of two cofactors: O^2 and erythrotetrahydrobiopterin. The activity of this enzyme can be antagonized by parachlorophenylalanine (PCPA) and has been used as a means to deplete 5-HT (Sanders-Bush et al., 1974). This paradigm has been instrumental in assessing the effects, or rather the lack, of 5-HT on anxiety and affective disorders (Goodwin and Post, 1974; Carlson, 1976) as well as efficacy of antidepressant treatments (see Delgado, 1999). Reserpine (a 5-HT releaser that subsequently depletes 5-HT intracellular stores) has also been used to assess the impact of this monoamine in depression and antidepressant efficacy (Mendels and Frazer, 1974; Price et al., 1987). Because only a small amount of L-tryptophan is able to penetrate the brain, the amino acid constitutes the rate-limiting step in 5-HT synthesis. Next, and in the presence of vitamin B6, decarboxylation of 5-hydroxytryptophan by the enzyme L-amino acid decarboxylase occurs, yielding 5-HT. The catabolism of 5-HT is performed by MAO to produce 5-hydroxyindoleacetaldehyde that is further oxidized to 5-hydroxyindoleacetic acid (5-HIAA). Catecholamines and 5-HT can be catabolized by the two isoforms of MAO (MAO-A and MAO-B) under certain conditions (Johnston, 1968) for which selective inhibitors have been developed. The oxidative deamination of 5-HT as well as NA and epinephrine is preferentially carried-out by the A isoform (abundant in the LC), whereas the

MAO-B form (abundant in the raphe) preferentially deaminates phenylethylamine and benzylamine. DA is deaminated by both forms (Westlund et al., 1985; Denney and Denney, 1985; Saura et al., 1992).

The first detailed anatomical map of the 5-HT system in a mammalian brain was provided by Dahlstrom and Fuxe, 1964, utilizing a method of capturing (freeze drying) 5-HT in tissue in combination in order to allow its detection by fluorescence microscopy. This is known as the Falck-Hillarp method. This initial technique, and others over the years, have allowed the 5-HT in the brain to be localized to the central gray, in the surrounding reticular formation, and in cell clusters located in the center, thus adopting the name *raphe*, from the Latin word for midline. Due to the high proportion of 5-HT neurons located in the raphe nuclei, as well as most of the studies pertaining to the body of work presented in this document being reference to, this is the only 5-HT cell population that will be elaborated on.

The raphe nuclei consists of a dense cluster of 5-HT cells that is subdivided into nine groups (B1-B9). Its numerical order corresponds to the cells' caudal to rostral orientation (Dahlstrom and Fuxes, 1964). The B1-B4 raphe nuclei are commonly referred to as the Caudal Linear Nucleus (CLN; Azmitia and Gannon, 1986; Tork, 1990) and extend along the rostral boundary of the superior cerebellar decussation. The depth of this structure is defined as ventrally bordering the interpeduncular nucleus, while the dorsal limit is the dorsal raphe nucleus (DR; B6 and B7). The former limit of the CLN is sometimes viewed (Lorez et al., 1978) as an extension of the median raphe nucleus (MRN; B5 and B8), but because of morphologic variations and these neurons' projections to different terminal fields, this is unlikely to be the case. The DR is the largest brainstem 5-HT nuclei and contains approximately 50% of the total 5-HT neurons in the mammalian CNS, whereas the MR comprises of 5% (Wiklund and Bjorklund, 1980; Descarries et al., 1982). The DR is not as homogenous of a structure for 5-HT neurons as that of NA neurons in the LC. The DR is primarily composed of 5-HT cells (70%) with the remaining being various peptidergic and non-peptidergic neurotransmitters containing neurons (Moss et al., 1983; Glazer et al., 1981; Beitz, 1982; Descarries et al., 1986).

These 5-HT neurons possess a regular discharge pattern resulting from a pacemaker cycle attributed to a Ca^{2+} -dependent K^+ outward current. The depolarization is followed by a long after-hyperpolarization period, which diminishes slowly during the interspike interval. During the depolarization, extracellular Ca^{2+} enters the neuron via a voltage-dependant Ca^{2+} channel activating a K^+ outward conductance, leading to an AHP. Ca^{2+} is then sequestered/extruded and the after-hyperpolarization period diminishes slowly. When the membrane potential reaches the low-threshold Ca^{2+} conductance, a new action potential is triggered (Aghajanian and Lakoski, 1984; Burlhis and Aghajanian, 1987; Aghajanian et al., 1990).

Table A14.9a
 Controlled Baseline Studies of CSF 5-HIAA in Depression and Mania

Study	Control	Patients N		50	Control Mean %	
		Depressed	Manic		100	150
Med-free ≥ 10 days						
Ashcroft et al.,1966	21	24		◆ ^a	19.1	
Papeschi & McClure,1971	10	12			◆	
Brodie et al.,1973	6	7		◆	28.0	
Goodwin et al.,1973	29	58	16		◆	○
Berger et al.,1980	23 M	13 M			27.3	◆
Banki et al.,1981	32 F	33 F			27.7	
Koslow et al.,1983	29 M	49 M	9 M		27.0	○
	29 F	43 F	5 F		20.1	◆ ^a ○ ^b
Roy et al.,1985b	41	27			21.0	◆
					13.0	
Widerlov et al.,1988a	10	22			◆	
Potter et al., unpublished data	49	100			15.0	◆
					15.6	
Widerlov et al, 1988 c	10(72.2+/-5.7)	22(70.3+/-5.0)			ns	

Geraciotti et al, 1997	10(47.7+-2.6)	10(46.2+-1.4)	pmol/ml (3 Bipolar)	ns
De Bellis et al 1993 d	46(111.2+-44.5)	9(95.9+-24.6)	pmol/ml	ns

Med-free<10 days

Bowers et al.,1969	8	8	8	39.5	
Van Praag & Korf, 1971	11	14		42.8	◊ ^a
Coppenetal.,1972b	20	31	18	40.0	◊ ^{a,b}
Wilk et al.,1972	19	5	6	42.3	◊ ^o
Van Praag et al.,1973	12	28		29.0	◊
Takahashi et al., 1974	30	30		30.4	◊ ^a
Subrahmanyam,1975	12	24		40.2	◊
Ashcroft et al.,1976	30	11 UP	11	16.0	◊ ^a
		9 BP			◊
Banki,1977	32 F	71 F	10 F	27.5	◊ ^{ob a}
Vestergaard et al., 1978	22	28	4	28.0	◊
Curzon et al.,1980	5	20		16.4	◊
Oreland et al.,1981	28M	6M			◊

Study	UP N	BP N	Control Mean (%)	UP Mean (%)	BP Mean (%)
Gerner et al., 1984	14 F	14 F	37	22.8	22.2
Asberg et al., 1984	66	60 UP 8 BPI 15 BPII		19.3	24.4
Gjerris et al., 1987	10	21		16.8	22.8

The mean metabolite level (ng/ml) for the control group in each study appears in the 100% column. Shaded bar indicates standard error (expressed in percentage points) around the control mean expressed as 100%. (Data not available in Wilk et al, 1972 and Gjerris et al., 1987)

◆ = Mean for depressed group expressed as % of control mean

○ = Mean for manic group expressed as % of control mean

^aDepressed vs. controls, p < .05

^bManics vs. controls, p < .01

^cPositive correlation between CRF and 5-HIAA

^dAfter fluoxetine treatment 5-HIAA to 64.2±26.1

Table A14.9b

CSF Metabolites in Unipolar versus Bipolar Depressed Patients

Study	Patients N		UP Mean =		
	UP	BP	50%	100%	150%

MHPG

Med-free ≥ 10 days

Goodwin & Post,1975	36	27 BP I 20 BP II	◆ ◆	11.0
Koslow et al.,1983	61	38	◆	8.9
Potter et al., unpubbshed data	54	47	◆	7.6
Swam et al, 1994 g	47	19		
Med-free < 10 days				
Vestergaard et al., 1978	13	6	◆	14.0
Agren,1980	21	12	◆	10.8
Asberg et al.,1984	26	2 BP I 6 BP II	◆	
Sheline et al, 1997 d	24			16.01+-17.23
HVA				
Med-free ≥ 10 days				
Ashcroft & Gilen, 1974	11	9	◆	20.0
Goodwin & Post, 1975	36	27 BP I 20 BP 20 BP II	◆	22.0
Bowers & Heninger, 1977 ^C	10	8	◆	136.0
Korf et al.,1983	17	17	◆	100.7
Koslow et al., 1983	26 M 32 F	23 M 11 F	◆	
Potter et al., unpublished data	54	47	◆	26.9

Tandon et al, 1988 h	14	28	mania 23.7+-3.9 mixed 20.2+-10.1	16.9+-14.3
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Roy et al, 1989	27	suicide reattempters had \downarrow 5-HIAA in 5 years follow-up study.		
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Med-free < 10 days

Banki,1977	55 F	16 F	\blacklozenge^b	24.0
Vestergaard et al., 1978	14	6	\blacklozenge	88.0
Agren,1980	21	12	\blacklozenge	37.1
Kasa et al.,1982	10	3	\blacklozenge	19.6
Asberg et al.,1984	43	4 BP I 11 BP II	\blacklozenge	36.7
Sheline et al, 1997 e	24			35.87+-15.93

5-HIAA

Med-free \geq 10 days				
Ashcroft&Glen,1974	11	9	\blacklozenge^a	10.0
Goodwin & Post,1975	36	27 BP I 20 BP II	\blacklozenge	26.0
Bowers&Heninger,1977C	10	8	\blacklozenge	111.8
Korf et al.,1983	17	17	\blacklozenge	65.2
Koslow et al.,1983	26 M	23 M	\blacklozenge	19.1
	31 F	12 F	\blacklozenge	

Potter et al., unpublished data	54	46	24.7 ◆ 16.7
Roy et al, 1989	27	suicide reattempters had 5-HIAA in 5 years follow-up study.	↓
Mann 1997	22	suicidal attempters	↓ 5-HIAA in high lethality attempters
Med-free < 10 days			
Banki,1977	55 F	16 F	◆ 16.4
Vestergaard et al., 1978	14	6	◆ 32.0
Agren, 1980	21	12	◆ 20.4
Asberg et al., 1984	60	8 BP I 15 BP II	◆ ◆ 17.1
Tandon et al, 1988	h	14	28
			↑ in mania vs mixed
Nordstrom 1994	92	1 year follow-up,	↓ 5-HIAA predicts short rank suicide attempt
Sheline 1997	f	24	14.54+-16.11

Mean metabolite level (ng/ml) for the unipolar group in each study appears in the 100% column. The shaded bar indicates standard error (expressed in percentage points) around the control mean expressed as 100%.

◆ = mean for bipolar depressed patients expressed as a percentage of the unipolar mean

^aUP < BP, *p*, .05

^bBP < UP, *p* < .01

^cStudy measured post-probenecid accumulations

^dDecreased MHPG after (7.76+-2.15) ng/ml vs. before (16.01+-17.23) treatment with fluoxetine and fluvoxamine *p*,0.05

^eTrend towards decreased HVA after (29.82+-11.57) vs. before (35.87+-15.93) treatment with fluoxetine and fluvoxamine. Ns.

^fDecreased 5-HIAA after (7.76+-2.15) vs. before (14.54+-16.11) treatment with fluoxetine and fluvoxamine *p*,0.05

g manic mixed 8, mania 11, agitated depression 20, non agitated depression 27. CSF values not shown.

Increase in MHPG in mixed manic vs agitated depression

h Bip: 14 mania, 14 mixed patients

Table A14.9c

Postmortem Brain Studies of Monoamine Function

Study	Subjects N		5-HT	5-HIAA	NE	DA	MHPG
	Depressed	Control					
Shaw et al., 1967	11	17	↓				
Bourne et al., 1968	16	15	NC	↓	NC		
Pare et al., 1969	23	15	↓	NC	NC	NC	
Lloyd et al., 1974	5	5	NC ^a	NC			
Baskow et al., 1976	11	62	NC	NC ^b	NC ^b	NC	
Cochran et al., 1976	10	12	NC				
Crow et al., 1984	15	22		NC			NC
Cheetham et al., 1989	19	19	↓	↑			
Young et al., 1994	9 ^e		↓ ^f	↓		↓ ^g	

Adapted from Crow et al., 1984; Goodwin and Jamison, 1990

NC = no change

^a 5-HT significantly reduced in 2 (dorsal raphe and central inferior nuclei) of 14 structures examined

^b NE reduced in 2 of 3 structures examined and 5-HIAA reduced in 6 of a structures examined, but significance attributed to time lapse until autopsy

^c in putamen

^d in amygdala

^e bipolar depressed

^f 5HT/5HIAA

^g DA and HVA/DA

5-HT Receptors

In 1957, the existence of two separate 5-HT receptors was first proposed, primarily due to the opposing phenomenon this neurotransmitter produces in relation to cholinergic mediation of smooth muscle contraction (Gaddum and Picarelli, 1957). Today, based on radioligand binding, signal transduction, and amino acid sequences, we know that 5-HT effectors are comprised of seven distinct receptors (5HT₁₋₇). The subtypes 5-HT_{1A,B,D,E,F} are negatively coupled to adenylate cyclase, while 5-HT_{2A,B,C} subtypes are positively coupled to PLC. 5-HT₃ receptors are the only fast-mediated excitatory 5-HT receptors, and are coupled to a ligand-gated ion channel. The 5-HT_{4,5,6,7} subtypes are positively coupled to adenylate cyclase (Humphrey et al., 1993). In relation to the present material, only the 5HT₁ to 5-HT₂ receptors will be discussed in detail, as these receptors more than the others have been implicated in psychiatric disorders.

5-HT₁ Receptors

Shortly after identification of the first two receptor subtypes (Peroutka and Snyder; 1981), Pedigo et al., (1981) identified the 5-HT_{1A} receptors with the use of spiperone, a drug that possesses a high and low affinity for the 5-HT_{1A} and 5-HT_{1B} binding sites, respectively. Following this, synthesis of the tetraline derivative 8-OH-DPAT was characterized as the first selective 5-HT_{1A} receptor agonist (Gozlan et al., 1982; Hjorth et al., 1989), which is now acknowledged to possess affinity for the 5-HT₇ receptor as well (see Hoyer et al., 1994). Since then, many other tetraline derivatives have effectively labeled the 5-HT_{1A} receptors, however, most possess only partial agonistic properties in postsynaptic

structures (Smith and Peroutka, 1986; Martin and Mason, 1987; Gartside et al., 1990; Yocca, 1990; Van der Hoof and Galvan, 1991; Blier and de Montigny, 1987). On the other hand, buspirone and ipsapirone are regarded as full agonists, but are plagued with sharing a common metabolite, 1-pyrimidinyl-piperazine (1-PP). This metabolite is a potent α_2 -adrenoceptor antagonist. Recently, 3-OH-gepirone (a metabolite of gepirone) has been indicated to act as a full agonist in some brain structures (Blier et al., 2000).

Binding and autoradiography experiments indicate that 5-HT_{1A} receptors throughout the brains of various species possess a high density in many limbic structures, including the hippocampus, septum, amygdala, and entorhinal cortex, as well as 5-HT neurons of the dorsal and median raphe (Marcinkiewicz et al., 1984; Pazos and Palacios, 1985; Welner et al., 1989; Hall et al., 1985; Waeber et al., 1989). The highest labeling is found in the DR with lower densities observed in the remaining raphe nuclei (Pazos and Palacios, 1985; Weissmann-Nanopoulos et al., 1985; Verge et al., 1985; Verge et al., 1986; Hensler et al., 1991; Pompeiano et al., 1992; Li et al., 1997). In the abovementioned experiments, all 5-HT_{1A} receptors were associated with being at least 50% in the raphe nuclei. This was shown by demonstrating that a selective degeneration of 5-HT neurons by intracerebral injection of the 5-HT neurotoxin 5,7-DHT is associated with a significant loss of 5-HT_{1A} binding only in the raphe nuclei. This is consistent with [³H] 8-OH-DPAT labeling a high amount of mRNA coding for the 5-HT_{1A} receptor, and a hybridization signal for this receptor subtype in the DR becoming abolished following a 5,7-DHT lesion (Pompeiano et al., 1992). Given that in postsynaptic areas the labeling for 5-HT_{1A} receptors correlate well with the presence of a hybridization signal for mRNA suggests further that the location of the 5-HT_{1A} receptors are somatodendritic in most regions (Pompeiano et al., 1992). On the other hand, labeling for the 5-HT_{1A} receptor in the CA₃ region of the hippocampus is low and corresponds to most of this receptor subtype being found in dendrites of pyramidal neurons (Pompeiano et al., 1992).

Through the use of molecular biology techniques, the 5-HT₁ receptor subtype has been shown to be coupled to multiple G-proteins. The 5-HT_{1A} receptor subtype inhibits adenylate cyclase via pertussis toxin-sensitive G_i proteins of which it is preferentially coupled (de Vivo and Maayani, 1985; Okada et al., 1989). Consistent with this observation, in cultured transfected cells, 5-HT in the nM range is able to inhibit the forskolin-stimulated adenylate cyclase activity (Fargin et al., 1989; Albert et al., 1990) and paradoxically can stimulate IP₃ and PKC production (Claustre et al., 1988; Raymond et al., 1989; Liu and Albert, 1991). It has been well demonstrated that the suppressant effect of 5-HT_{1A} receptor

activation on DR and dorsal hippocampus firing activity is mediated by a pertussis toxin-sensitive mechanism that does not require a second messenger (Innis and Aghajanian, 1987; Innis et al., 1988; Andrade et al., 1986).

Postsynaptic 5-HT_{1A} receptors are also abundantly expressed by astrocytes and some other glia. These 5-HT_{1A} receptors are expressed by the majority of astrocytes in frontal and limbic cortex, but essentially none of the astroglia in striatum, thalamus, or cerebellum. Stimulation of astrocyte-based 5-HT_{1A} sites causes astrocytes to acquire a more mature morphology and to release the trophic factor, S-100B, which promotes growth and arborization of serotonergic axons. S-100B is primarily released by astroglia in the developing brain, when it plays a role in the development of the serotonergic system. S-100B also plays a role in maintaining the cytoskeleton in adult animals by promoting tubulin polymerization and inhibiting PKC-mediated breakdown of microtubules. In addition, stimulation of neuron-based 5-HT_{1A} receptors inhibits PKA-mediated disassociation of the proteins comprising the tubulin polymers of the cytoskeleton. Administration of 5-HT_{1A} receptor antagonists, antibodies to S-100B, or agents that deplete 5-HT all produce similar losses of dendrites, spines and/or synapses in adult and developing animals, effects which are blocked by administration of 5-HT_{1A} receptor agonists or SSRIs. The role of postsynaptic 5-HT_{1A} receptor function in maintenance of the cytoskeleton has led to the hypothesis that a reduction of 5-HT_{1A} receptor function may comprise a risk factor for the neuropathological abnormalities identified in limbic and paralimbic cortical areas in mood disorders (reduced cortex volume, reduced synaptic proteins, increased neuronal density, reduced glial counts).

Regulation of 5-HT_{1A} Receptor Expression

The density and mRNA expression of 5-HT_{1A} receptors appear insensitive to reductions in 5HT transmission associated with lesioning the raphe or administering the serotonin depleting agent, PCPA. Similarly, elevations of 5HT transmission resulting from chronic administration of SSRI or monoamine oxidase inhibitors (MAOI) do not consistently alter 5-HT_{1A} receptor density or mRNA in the cortex, hippocampus, amygdala, or hypothalamus. In contrast to the insensitivity to [5HT], 5-HT_{1A} receptor density is down-regulated by adrenal steroids. Postsynaptic 5-HT_{1A} receptor gene expression is under tonic inhibition by adrenal steroids in the hippocampus and some other regions. Thus, in rodents the hippocampal 5-HT_{1A} receptor mRNA expression is increased by adrenalectomy and decreased by corticosterone (CORT) administration or chronic

stress. The stress-induced down-regulation of 5-HT_{1A} receptor expression is prevented by adrenalectomy. Mineralocorticoid receptor stimulation has the most potent effect on down-regulating 5-HT_{1A} receptors, although glucocorticoid receptor stimulation also contributes to this effect.

5-HT_{1D} receptors are virtually absent in the rodent but detected in guinea pig and man (Bruinvels et al., 1993). It has been proposed that 5-HT_{1B} receptors are the rodent homologue of 5-HT_{1D} receptors (see Saxena et al., 1998). The 5-HT_{1D} subtype shares a modest homology of 74% with the 5-HT_{1B} receptors (Hamblin et al., 1992; Weinshank et al., 1992). In light of this, most pharmacological agents have not been able to differentiate between the two and possess a similar affinity for both subtypes. Also, the distribution of the 5-HT_{1D} receptors in guinea pig and man are roughly equivalent to 5-HT_{1B} receptors in the rat (Bruinvels et al., 1993). This is concordant with electrophysiological data from several laboratories, implicating that 5-HT_{1D} autoreceptors mediate a negative feedback influence on the release of 5-HT (Cerrito and Raiteri, 1979; Martin and Sanders-Bush, 1982; Gothert and Weinheimer, 1979). Similar to their 5-HT_{1A} somatodendritic autoreceptor counterparts, 5-HT_{1D} receptors are also negatively coupled to adenylate cyclase. Both the 5-HT_{1B} and 5-HT_{1D} receptors have been demonstrated in various models to inhibit the stimulation of forskolin-mediated cAMP (Hamblin et al., 1992; Zgombick et al., 1993; Hoyer et al., 1990; Weinshank et al., 1992; Schoeffter and Hoyer, 1989). Activation of 5-HT_{1B} and 5-HT_{1D} receptors stimulates PLC that then elevates intracellular Ca²⁺ (Zgombick et al., 1993).

The 5-HT_{1D} receptors are also located on 5-HT neurons in the rat DR and modulate the release of 5-HT in this nucleus (Hamblin et al., 1992; Piñeyro et al., 1995). This was experimentally deduced, as the preferential 5-HT_{1B} agonist CP 93,129 in a concentration-dependent manner inhibited the electrically-evoked stimulation of [³H]5-HT in preloaded hippocampus slices, and was without effect on mesencephalic slices containing DR 5-HT neurons (Piñeyro et al., 1996). These results implied that the autoreceptor in the raphe was not of the 5-HT_{1B} subtype. Furthermore, in hippocampus slices prepared from 5-HT_{1B} knockout mice, CP 93,129 did not inhibit evoked 5-HT overflow, in contrast to its marked suppressant effect in wild type mice (Piñeyro et al., 1995). It has also been demonstrated that terminal 5-HT_{1B} autoreceptors are not coupled to G-proteins (Blier, 1991), but in the rat substantia nigra they do in fact inhibit forskolin stimulation of adenylate cyclase (Bouhelal et al., 1988; Schoeffter and Hoyer, 1989). Thus, different receptor coupling mechanisms may vary according to brain region.

5-HT₂ Receptors

There are three subtypes of 5-HT₂ receptor, denoted as 5-HT_{2A,B,C}. The highest level of 5-HT_{2A} binding sites and mRNA for these receptors exists in the cortex, and they are implicated in the production of hallucinations with psychomimetic agents (for review see Aghajanian and Marek, 1999). In addition, 5-HT neuron lesions with 5,7-DHT did not reduce the 5-HT₂ receptor density reported in brain regions (Hoyer et al., 1986; Fischette et al., 1987; Conn et al., 1987; Hoffman and Mezey, 1989; Pompeiano et al., 1994; Wright et al., 1995) This indicates that these receptors are located postsynaptically. Recently, MDL 100,907 has been identified as a selective and potent 5-HT_{2A} receptor antagonist (Sorensen et al., 1993; Johnson et al., 1996; Kehne et al., 1996). Autoradiography with [³H]MDL 100,907 has localized 5-HT_{2A} receptors to many similar brain regions in the rat and primate brain (Lopez-Gimenez et al., 1998). One of these brain regions is the LC (Lopez-Gimenez et al., 1999, 2001). Until recently, no selective ligands for the 5-HT_{2C} receptors (formerly denoted as 5-HT_{1C}) were available. Competitive studies with other radioligands (Yagaloff and Hartig, 1985; Sanders-Bush and Breeding, 1988; Westphal and Sanders-Bush, 1994) and its mRNA distribution indicate 5-HT_{2C} receptors as being considerably widespread through the CNS, with the highest density in the choroid plexus (Hoffman and Mezey, 1989). 5-HT_{2C} receptors have been detected in both the DR and LC (Molineaux et al., 1989; Pompeiano et al., 1994; Wright et al., 1995; Abramowski et al., 1995), but nowhere in the brain are 5-HT_{2B} receptors detected (Pompeiano et al., 1994; Hoyer et al., 1994).

All of the 5-HT₂ receptor subtypes are linked to the phosphoinositide (PI) signaling system, and their activation produces inositol triphosphate (IP₃) and diacylglycerol, via PLC activation (Conn and Sanders-Bush, 1987; Conn et al., 1987; Launay et al., 1994). Several tritiated ligands, such as spiperone, ketanserin, mianserin, metergoline [¹²⁵I] LSD, and [¹²⁵I] ketanserin have been used to describe 5-HT₂ receptors, as well as agonists such as DOB and DOI (Titeler et al., 1987; McKenna and Peroutka, 1989). In addition to the display of these 5-HT₂ receptors, it has been demonstrated that agonist binding induces a rapid internalization (Willins et al., 1998). This would be equivalent to an antagonistic-like effect and represents an important issue to consider when evaluating the mechanism of action of antidepressants.

5-HT Transporters

Termination of 5-HT in the synaptic cleft includes degradative metabolism of enzymes; it also includes the ability of 5-HT transporter (5-HTT) to remove 5-HT from the synaptic cleft by an ion-dependent reuptake process. 5-HT is taken up into the presynaptic terminals where it is metabolized by MAO or sequestered into secretory vesicles by the vesicular transporter. The cDNA for the brain 5-HT transporters (5-HTT) has been cloned from rat (Blakely et al., 1991; Hoffman et al., 1991), mouse (Chang et al., 1996) and human (Lesch et al., 1993; Ramamoorthy et al.,

1993). Cloning and sequencing of cDNA-encoding 5-HTT has revealed two related proteins with twelve transmembrane domains (similar to that of the NATT) containing the secondary structure required for the substrate translocation, ion, and antagonist binding (Blakely et al., 1991; Hoffman, 1994). 5-HTT is located outside of the CNS in the periphery, being produced by enteric 5-HT neurons (Wade et al., 1996) as well as non-neuronal cells, such as mast cells (Gripenberg, 1976), cript epithelial cells, and enterochromaffice cells (Wade et al., 1996). 5-HTT is also located in platelets (Rudnick, 1977; Quian et al., 1995), lung membranes (Quian et al., 1995) and maternal brush-border of syncytiotrophoblasts (Cool et al., 1990; Ramamoorthy et al., 1993).

In the brain, 5-HTTs have been radiolabeled with [³H] imipramine (Langer et al., 1980; Dawson and Wamsley, 1983; Hrdina et al., 1985) and more selectively with 5-HT uptake inhibitors such as [³H]cyanoimipramine (Wolf et al., 1988; Kovachich et al., 1988; Soucy et al., 1994), [³H]paroxetine (Habert et al., 1985; de Souza and Kuyatt, 1987; Langer et al., 1987; Marcusson et al., 1988) and [³H] citalopram (D'Amato et al., 1987). [³H] imipramine appears to be similar to [³H]SSRIs (Hrdina et al., 1990; Duncan et al., 1992), but regional differences in density have been detected. The former yields a much higher density for binding in the forebrain areas such as cortex and hippocampus. This is presumably due to [³H]imipramine being able to bind to two classes of sites on the 5-HTT, being the high and low affinity sites. However, only the high affinity sites seem to be related to 5-HT uptake (Moret and Briley, 1986; Marcusson et al., 1986; Hrdina 1987, 1988; D'Amato et al., 1987). Many SSRIs have been radiolabeled, however, due to low specific to non-specific binding ratios. Paroxetine (Arranz and Marcusson, 1994) and citalopram (Descarries et al., 1995) are regarded as optimal for in vitro studies.

Cellular localization of 5-HTT in the CNS has been accomplished by using site-specific antibodies (Lawrence et al., 1995a, b; Qian et al., 1995). Immunocytochemistry directed against sites on the second and third intracellular loops of the 5-HT carrier revealed both neuronal and glial staining in areas of the rat brain containing 5-HT somata and terminals (i.e., DR and hippocampus; Lawrence et al., 1995b). 5-HT uptake ability has been documented in primary astrocyte cultures (Katz and Kimelberg, 1985; Kimelberg and Katz, 1985) and accounts for 50% - 80% in the frontal cortex and periventricular region, respectively (Anderson et al., 1992). It is hypothesized that the first step in 5-HT transport involves the binding of 5-HT to the 5-HTT and then a co-transport with Na⁺, while the second step involves the translocation of K⁺ across the membrane to the outside of the cell. SSRIs bind to the same site on the transporter as 5-HT itself. The regional distribution of 5-HTT corresponds to discrete regions of rat brain known to contain cell bodies of 5-HT neurons and synaptic axon terminals (Backstrom et al., 1989; Hrdina et al., 1990; Mann and Hrdina, 1992).

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