0031-6997/99/5103-0533$03.00/0
PHARMACOLOGICAL REVIEWS
Vol. 51, No. 3

Printed in U.S.A.

Autoregulation of Serotonin Neurons: Role in Antidepressant Drug Action

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This paper is available online at http://www.pharmrev.org

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I. Introduction

The existence of an endogenous vasoconstrictor in blood serum (Stevens and Lee, 1984; Brodie, 1900) and the presence in the gut of a substance that increases intestinal motility (Vialli and Ersparmer, 1933) had been known to scientists since the beginning of the century. However, it was not until the serum vasoconstrictor was identified as 5-hydroxytryptamine (5-HT)[2] (Rapport et al., 1948) that it became clear that this amine was also present in the mammalian central nervous system (CNS) (Twarog and Page, 1953). Shortly after its discovery in the CNS, and based on the observation that it was heterogeneously distributed in the dog brain (Bogdansky et al., 1956), 5-HT was considered for the first time a putative neurotransmitter in the CNS. A major turning point in 5-HT neurotransmission research then came about when Fuxe and Dahlström, using Falck-Hillarp histochemical fluorescence, provided the first description of 5-HT neurons (Dahlström and Fuxe, 1964) and their projections (Fuxe, 1965). Today, it is an established fact that no region in the mammalian CNS lacks 5-HT innervation (Dahlström and Fuxe, 1964; Steinbusch, 1981, 1984; see Azmitia, 1986; Jacobs and Azmitia, 1992; Azmitia-Whitaker and Azmitia, 1995).

As a neurotransmitter, the ubiquity of 5-HT is not only anatomical but also phylogenetic. Having been identified in neurons of the cnidarian *Renilla koellikeri* (Umbriaco et al., 1990), it could be one of the most ancient of currently known transmitters. From primates (see Azmitia and Gannon, 1986; Törk, 1990) to *Chondrichtyes* (Stuesse and Cruce, 1992), the adult 5-HT system is organized into two subsystems, i.e., a rostral division with cell bodies localized in the midbrain and rostral pons, providing projections to the forebrain, and a caudal division located primarily in the medulla oblongata with descending projections to the spinal cord and brainstem nuclei. Similarities found among such divergent vertebrate brains indicate that the major nuclear organization and the 5-HT projection network have remained remarkably stable across phylogeny. In spite of its remarkable evolutionary stability (Jacobowitz and MacLean, 1978), careful scrutiny of comparative anatomical evidence indicates that differences are as important as similarities. These phylogenetic differences may be summarized as follows: in higher mammals the system has evolved toward a fast, precise type of neurotransmission in which 5-HT neurons give rise to few collaterals (Fallon and Loughlin, 1982), have a high proportion of myelinated axons, and, due to the existence of a high proportion of junctional synapses, terminal field innervation is localized. In lower mammals the system is diffuse, highly branched, unmyelinated, and nonjunctional innervation predominates in terminal fields (see Azmitia 1986; Descarries et al., 1990; Törk, 1990; Jacobs and Azmitia, 1992; Azmitia and Whitaker-Azmitia, 1995).

Because 5-HT neurons are among the first neuroblasts to differentiate, the 5-HT system is also ontogenically ancient. Indeed, in the rat brain 5-HT-immunoreactivity appears as early as gestational day 12 (Olsen and Seiger, 1972; Lidov and Molliver, 1982), tryptophan hydroxylase activity is present at fertilization, and nearly every embryonic cell contains 5-HT until the gastrula stage (Harris, 1981). The first 5-HT neurons appear in early development and are located rostrally, within the mesencephalon (Lidov and Molliver, 1982) Two days later, a more caudal rhombencephalic group appears (Wallace and Lauder, 1983). In the next sections, focus will be on the rostral group.

II. Morphological Aspects of the 5-HT Rostral System

A. 5-HT Nuclei

Dahlström and Fuxe (1964) divided 5-HT cell clusters into nine groups (B_{1-9}), B_1 being the most caudal group of cells. Today, however, the nomenclature most frequently used refers to 5-HT cells contained within cytoarchitectonic brainstem entities known as the raphe nuclei. Rostral 5-HT neurons are not confined to midline (raphe) nuclei, they are also present in more lateralized sites of the reticular formation, especially 1) dorsal to the medial lemniscus (rat: Dahlström and Fuxe, 1964; Baker et al., 1990) and 2) dorsal to the nucleus raphe pontis oralis (Lidov and Molliver, 1982; Baker et al., 1991).

[2] Abbreviations: 5-HT, 5-hydroxytryptamine; CNS, central nervous system; VTA, ventrotegmental area; bFGF, basic fibroblast growth factor; ACTH, adrenocorticotrophic hormone; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,3-methylenedioxymethamphetamine; PCA, *p*-chloroamphetamine; GPCR, G protein-coupled receptor; REM, rapid eye movement; NMDA, *N*-methyl-D-aspartate; EPSP, excitatory postsynaptic potential; 5-MeOT, 5-methoxytryptamine; TTX, tetrodotoxin; PKC, protein kinase C; CYP, cytochrome P-450; PAPP, 4[2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethyl]benzeneamine; ACh, acetylcholine; CCK, cholecystokinin; CLN, caudal linear nucleus; DA, dopamine; DRN, dorsal raphe nucleus; EAA, excitatory amino acid; ECS, electroconvulsive shock; GABA, γ-aminobutyric acid; GTI, serotonin-5-*O*-carboxymethyl-glycyl; LSD, lysergic acid diethylamide; MAOI, monoamine oxidase inhibitor; MLF, medial longitudinal fasciculus; MRN, median raphe nucleus; NE, norepinephrine; NEM, *N*-ethylmaleimide; PCPA, *p*-chlorophenylalanine; SSRI, selective serotonin reuptake inhibitor; SERT, serotonin transporter; SWS, slow wave sleep; TFMPP, 1-[3-trifluoromethyl)phenylpiperazine; VIP, vasointestinal polypeptide; 5-CT, 5-carboxyamidotryptamine; 5-HIAA, 5-hydroxyindole acetic acid; 5-HTP, 5-hydroxytryptophan; 5,7-DHT, 5,7-dihydroxytryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; PET, photon emission tomography.

1. The Caudal Linear Nucleus. The caudal linear nucleus (CLN), described in primates by Törk (1990) and Azmitia (see Azmitia, 1986; Azmitia and Gannon, 1986; Jacobs and Azmitia, 1992; Azmitia and Whitaker-Azmitia, 1995), is the most rostral group of serotonergic neurons in the mesencephalic midline, extending along the rostral boundary of the superior cerebellar decussation. Its ventral limit is defined by the interpeduncular nucleus and dorsally it contacts the dorsal raphe nucleus (DRN) through the gap left between the two medial longitudinal fasciculi. In rats, 5-HT neurons located rostral to the decussation of the superior cerebellar peduncle and above the interpeduncular nucleus have been considered in some cases a rostral extension of the median raphe nucleus (MRN) (Lorez et al., 1978; Parent et al., 1981). However, CLN and MRN neurons do not share common terminal projection fields (Imai et al., 1986) and display dissimilar dendritic morphology (Hornung and Fristchy, 1988). On the other hand, because DRN and CLN 5-HT neurons innervate similar terminal fields (e.g., caudate-putamen in adult rat brain; Imai et al., 1986) and share a common developmental origin (Wallace and Lauder, 1983), an alternative approach proposes that CLN neurons should be considered similar to those in DRN (see Jacobs and Azmitia, 1992).

2. The Dorsal Raphe Nucleus. The DRN is the largest of the brainstem serotonergic nuclei containing about 50% of 5-HT neurons in the rat CNS (Wiklund and Björklund, 1980; Descarries et al., 1982), 40% in the cat CNS (Wiklund et al., 1981), and 50 to 60% in the human CNS (Baker et al., 1990). Rostrally, the dorsal raphe is bound by the Edingher Westphal nucleus (III) and, caudally, it extends just ventral to the confluence of the fourth ventricle and the cerebral aqueduct (Steinbusch, 1981; Descarries et al., 1982; Imai et al., 1986; Törk, 1990; Jacobs and Azmitia; 1992). In most species, the DRN is composed of several subregions distinguished by their different cell density, morphology, and projections (Azmitia and Gannon, 1986; Baker et al., 1991; Johnson and Ma, 1993): 1) a medial portion, subdivided in turn into dorsomedial and ventromedial components, just below the cerebral aqueduct and surrounding the medial longitudinal fasciculus (MLF), respectively; 2) lateral portions or wings (much more prominent in primates than other mammals due to a lateralization process through phylogeny; Descarries et al., 1982; Azmitia and Gannon, 1986; Baker et al., 1991); and 3) a caudal component. During development (15 days of gestation), 5-HT-immunoreactive cells group themselves into two different clusters, dorsolateral and ventrolateral to the MLF (Wallace and Lauder, 1983). The dorsolateral portion will give rise to the lateral wings of the DRN, whereas the ventrolateral group will split to form the interfascicular portion of DRN and MRN. For this reason, it has been proposed that, in the primate CNS (see Jacobs and Azmitia, 1992; Azmitia and Whitaker-Azmitia, 1995), the interfascicular subregion of the DRN should be best considered an entity along with the MRN. Olzewski and Baxter (1954) had previously defined an anatomical entity, nucleus centralis superior, consisting of three groups of cells: 1) a dorsalis component situated between MLF, 2) a medialis component or MRN, and 3) a lateralis component which includes the 5-HT cells that form the ring around the nucleus reticularis pontis oralis (see Table 1). The absence of anatomical boundaries between the main rostral portion of the DRN (B_7) and the main rostral portion of the MRN (B_8) has also been observed in rats (Descarries et al., 1982) and cats (Wiklund et al., 1981). The main rostral component of the DRN (B_7) also merges, in its rear end, with the caudal component (B_6) of this same nucleus (see Jacobs and Azmitia, 1992; Azmitia and Whitaker-Azmitia, 1995). Furthermore, the latter caudal component is in continuity with the dorsocaudal portion of the MRN (see Jacobs and Azmitia, 1992; Azmitia and Whitaker-Azmitia, 1995) and the lateral wings of the DRN (Lidov and Molliver, 1982). Hence, anatomical proximity between different 5-HT cell clusters provides the ground for a network of homotypic interconnections among 5-HT neurons. Such interconnections constitute in turn the morphological ground for the high degree of cross-talk existing among 5-HT neurons at the somatodendritic level. The major implication underlying this arrangement is that the more closely knit and the larger the number of cells in a functional cluster, the more powerful will be the effect of the group on the target structure toward which it projects (Azmitia, 1986).

3. The Median Raphe Nucleus. The MRN consists of two distinct parts, i.e., a group of densely packed cells, vertically oriented and concentrated in the midline, and a second group, the paramedian columns, consisting of cells scattered in the periphery of the midline cluster (Köhler and Steinbusch, 1982). The nucleus is situated ventral to the MLF and has a rostrocaudal oblique orientation (Azmitia, 1981; Lidov and Molliver, 1982). In its rostral end, beyond the MLF, it is separated from the DRN in its rostral end by the superior cerebellar peduncle decussation. Its medial portion extends from the mesencephalic interpeduncular nucleus to the trapezoid body in the pons. Laterally, the limits of the nucleus are poorly defined toward the reticular formation (Köhler and Steinbusch, 1982), and in the rat the MRN merges laterally with the 5-HT group in nucleus raphe pontis oralis (Lidov and Molliver, 1982). The MRN forms the second largest cluster of 5-HT neurons in the mammalian CNS (Baker et al., 1990).

4. The Supralemniscal Region. The last group of 5-HT neurons to be considered corresponds to 5-HT cells in the supralemniscal region. In the rat, 5-HT cells belonging to this group are scattered along the dorsal border of the medial lemniscus and extend their dendrites in between the fiber bundles of the latter (Parent et al., 1981; Steinbusch, 1981). A similarity between supralemniscal 5-HT cells and those of the MRN was first noted by Dahlström

and Fuxe (1964) in the rat CNS and has been later confirmed in primates (Hubbard and Di Carlo, 1974). In fact, the cells in the supralemniscal region may be continuous with those of the paramedian columns of the MRN (Azmitia and Gannon, 1986). Unlike rodents in which the supralemniscal cell cluster is predominantly mesencephalic, in humans it is entirely located in the pons (Baker et al., 1991).

B. Ultrastructure

In all species studied thus far, neurons containing 5-HT consist of a morphologically heterogeneous population (Steinbusch, 1981; Jacobs et al., 1984; Azmitia and Gannon, 1986; Törk and Hornung, 1990). The average cell diameter varies between 15 and 25 μm (Azmitia, 1978; Descarries et al., 1982), and, ultrastructurally, "5-HT specificity does not closely correlate with any particular neuronal configuration and/or intracellular build-up" (Descarries et al., 1982). No unique element allows to distinguish 5-HT cell bodies from non-5-HT surrounding neurons.

The average number of axosomatic boutons received by 5-HT neuron perikarya in the DRN has been quantified by Descarries et al. (1982). Although 100 μm of somatic membrane receive seven axonic boutons, the average number of spines on the same membrane length is 2.7 (Park et al., 1982). On the dendrites, the number of axonic boutons per 100-μm membrane length is roughly 60% higher than on the soma (Descarries et al., 1982). In cats and rats, the fibers contributing to these axosomatic or axodendritic contacts are non-5-HT fibers (Descarries et al., 1982; Chazal and Ralston, 1987). In primates, Kapadia et al. (1985) have described 5-HT fibers impinging on 5-HT dendrites.

1. Sources of Extracellular 5-HT in Rostral Raphe Nuclei. The existence of serotonergic axon terminals in the raphe nuclei of cats and rats has been repeatedly reported (Baraban and Aghajanian, 1981; Chan-Palay, 1982; Descarries et al., 1982; Chazal and Ralston, 1987), but the terminals endowed with synaptic specializations have been found to be consistently in low numbers, ranging from "none" (Baraban and Aghajanian, 1981), "exceedingly small number" (Descarries et al., 1982), to "a few" (Chazal and Ralston, 1987). Furthermore, Baraban and Aghajanian (1981) found 5-HT fibers exclusively in axon bundles rather than in proximity to dendrites or cell bodies, and when 5-HT terminals were observed to make somatodendritic synaptic contacts, these were on non-5-HT neurons (Descarries et al., 1982; Chazal and Ralston, 1987). In the rare cases in which a 5-HT axon terminal was observed in close apposition to a 5-HT cell body, no demonstrable synaptic contact was present (Chazal and Ralston, 1987). Nonsynaptic 5-HT axon terminals are not exclusive to the raphe nuclei; they also exist in projection areas such as the cortex, striatum, and hippocampus (Descarries et al., 1990; Törk, 1990). 5-HT release therefore occurs not only from junctional but also from nonjunctional sites (Descarries et al., 1975, 1982, 1990).

The total number of 5-HT axons that reach the rostral raphe nuclei (independent of their synaptic specializations), as well as their origin, is a controversial issue in the literature. In autoradiographic studies of the rat brain, Descarries et al. (1982) reported "only few" [^{3}H]5-HT-labeled axon terminals reaching the DRN; Chan-Palay (1982) found "numerous" such terminals; whereas Baraban and Aghajanian (1981) reported prominent labeling of unmyelinated 5-HT axons. On the other hand, using an immunohistochemical procedure, Brusco et al. (1983) found only few 5-HT axon terminals in rat DRN, whereas Chazal and Ralston (1987) concluded that, in the cat, the latter are "widely distributed and not uncommon", although "not numerous as compared to non-5-HT terminals". Furthermore, the fine structure of fibers impinging on the DRN was similar to that of 5-HT fibers found in terminal projection areas (Beaudet and Descarries, 1981), a fact that should be borne in mind when considering whether 5-HT fibers that impinge on the rostral 5-HT nuclei are collaterals or afferents from other 5-HT nuclei. In primates, using immunocytochemistry, Kapadia et al. (1985) have also reported few 5-HT axon terminals in the DRN.

In four of the five above-mentioned studies, the number of 5-HT fibers impinging on the raphe nuclei was found to be scarce. However, the extracellular concentration of 5-HT at the somatodendritic level is twice that observed in projection areas such as the cerebral cortex (e.g., 5.5 and 2.3 nM, respectively; Bel and Artigas, 1992), where the amount of axon terminals is 5.8×10^6 varicosities/mm^3 (Descarries et al., 1990). It is then difficult to conceive that 5-HT fibers (collaterals or afferents from other nuclei) would be the only source of extracellular 5-HT in the dorsal raphe. An alternative and conceivably important source of extracellular 5-HT in the raphe area would therefore be the soma and dendrites of 5-HT neurons. Indeed, the existence of 5-HT neurons with vesicle-containing dendrites in the cat DRN has been reported (Chazal and Ralston, 1987). These dendrites may be divided into two different types: 1) dendrites in which vesicles were never found to be associated with any membrane specialization, and 2) vesicle-containing dendrites which have synaptic membrane specializations. It was not determined whether the two types of dendrites could belong to the same neuron. It is possible that the different types of dendrites could serve different functions: the ones lacking any junctional specialization could contribute to maintain high extracellular-extrasynaptic somatodendritic concentrations of 5-HT, whereas those bearing specializations could constitute the anatomical basis for dendrodendritic homotypic interactions between 5-HT neurons. In fact, although sometimes vesicle-containing dendrites were found to contact non-5-HT elements, most frequently they were presynaptic to other 5-HT

dendrites. Apposition between 5-HT dendrites has also been described in rats (Descarries et al., 1982), rabbits (Felten and Harrigan, 1980), and primates (Kapadia et al., 1985). Synaptic specializations on dendrodendritic contacts were recognized in cats (Chazal and Ralston, 1987), as well as in the primate brain (Kapadia et al., 1985), although 5-HT dendrites containing vesicles were found only in the cat CNS. Despite these vesicle-containing dendrites being present throughout the DRN, they were infrequent as compared to dendrites that did not contain vesicles. Furthermore, 5-HT dendrodendritic synapses constituted a small portion of the total DRN synapses (Chazal and Ralston, 1987). A similar observation has been made in the substantia nigra where only a few of the total dendrodendritic contacts interconnected dopaminergic neurons (Wilson et al., 1977). Still, K^+-induced depolarization evokes significant dopamine (DA) release from this region (Chéramy et al., 1983). A neurotransmitter-releasing role for 5-HT dendrites was initially proposed by Wang and Aghajanian (1977a, 1978) who observed that inhibition of 5-HT neuron firing following the stimulation of the ascending 5-HT pathway in the ventrotegmental area (VTA) was abolished by the 5-HT synthesis inhibitor *p*-chlorophenylalanine and restored by 5-hydroxytryptophan. It was also proposed that the latter inhibitory effect could be mediated by recurrent 5-HT axon collaterals. Since their initial reports (Wang and Aghajanian, 1977a, 1978), recurrent inhibition of 5-HT neuron firing activity has been confirmed using intracellular (Park et al., 1982) and extracellular (Piñeyro et al., 1996b) recordings. In the former study, the injection of horseradish peroxidase into a representative 5-HT neuron that showed recurrent inhibition following VTA stimulation revealed a single collateral arising from the neuron's axon. Although this observation supports the existence of axon collaterals, it is not evident that they constitute the only or even an important anatomical substrate for recurrent inhibition of 5-HT neuron firing activity. Moreover, two major Golgi studies in rat and rabbit (Felten and Cummings, 1979; Díaz-Cintra et al., 1981) described only a few axon collaterals. Summarizing the above-mentioned evidence, it seems reasonable to conclude that the main source of extracellular 5-HT within the raphe nuclei is of somatodendritic origin.

2. Cell Bodies Found in the 5-HT Nuclei. Quantitative studies of the total number of 5-HT neurons located in the ascending raphe nuclei indicate that there are about 288,000 in the human brain (Törk, 1990), 33,000 in the cat brain (Wiklund et al., 1981), and 15,200 in the rat brain (see Jacobs and Azmitia, 1992). Moreover, 5-HT neurons represent but a small percentage of the total neuronal population of the raphe nuclei. Using histofluorescence-imaging techniques, Wiklund et al. (1981) reported that in the cat 5-HT neurons constitute 70% of medium-sized cells in the dorsal raphe and 35% of medium-sized neurons of the MRN. This percentage could be lower, serotonergic cells constituting 25 to 50% of the total DRN neuronal population and 20 to 30% of the MRN′s. In the supralemniscal 5-HT cell group (B_9), the percentage of 5-HT neurons appears to be much lower than in the other two nuclei (O'Hearn and Molliver, 1984).

Non-5-HT cells reported in the mesencephalic raphe nuclei include peptidergic and nonpeptidergic neurons. The most numerous non-5-HT perikarya are the peptidergic enkephalin-immunoreactive cells (Uhl et al., 1979; Moss et al., 1981, 1983). Most of enkephalin and 5-HT immunoreactivity in dorsal and median raphe nuclei do not colocalize, thus indicating that enkephalin- and 5-HT-immunopositive cells should be best considered distinct neuronal populations (Tanaka et al., 1993). Other peptides contained in DRN somata include 1) Substance P, abundant in lateral wings (Moss et al., 1983; Magoul et al., 1986) and rostral portion of the nucleus (Ljungdahl et al., 1978). It is worth noting that in bulbospinal neurons Substance P reduces the affinity and increases the density of [^{3}H]5-HT-binding sites (Agnati et al., 1983), indicating that the neuropeptide released from 5-HT neurons may modulate receptor sensitivity to 5-HT; 2) neurotensin, found in cells dorsal to the MLF at mid- and caudal levels of the DRN (Beitz, 1982); 3) neurons positive for vasointestinal polypeptide (VIP), which are few and located just ventral to the aqueduct (Sims et al., 1980; Moss et al., 1983); and 4) somatostatin- and cholecystokinin (CCK)-positive cells, both found within the periaqueductal gray, but few of them lie within the DRN (Vanderhaeghen et al., 1980).

There is also consistent evidence indicating the existence of DA-positive cells in the midbrain raphe nuclei (Hökfelt et al., 1976; Ochi and Shimizu, 1978; Miachon et al., 1984; Trulson et al., 1985; Descarries et al., 1986). Except for Miachon et al. (1984) who reported few tyrosine hydroxylase-positive DA-β-hydroxylase-negative neurons, there is general agreement that DA cell bodies in the midbrain raphe are numerous. Two primary subpopulations of DA neurons have been described: 1) A_{10dc}, lying on the extreme dorsal border of the rostral half of the DRN, ventral to the cerebral aqueduct and 2) A_{10c}, occupying the medial aspect of the DRN and extending dorsocaudally from the ventrorostral border of this nucleus, where the cells appear contiguous with those of A_{10} (Hökfelt et al., 1984). Similar to A_{10} DA neurons, DA-containing cells in rat DRN project to nucleus accumbens (Stratford and Wirtshafter, 1989) and neostriatum (Descarries et al., 1986) with a low degree of collateralization. This projection pattern differs from that of surrounding 5-HT-immunoreactive neurons which show profuse colateralization and innervate other structures such as septum (de Olmos and Heimer, 1980), prefrontal cortex (O'Hearn and Molliver, 1984; Waterhouse et al., 1986; Imai et al., 1986), and neostriatum (Jacobs et al., 1978; Imai et al., 1986). Stratford and Wirtshafter (1989) have thus suggested that DRN DA

cells represent a caudal extension of A_{10} and hence a completely different population from 5-HT neurons. Furthermore, no colocalization of 5-HT and tyrosine hydroxylase was observed in the raphe nuclei (Vanhatalo et al., 1995). Norepinephrine-positive cells have not been found within the DRN, but are located just caudal to the lateral wing groups at the limits of the locus ceruleus (Grzanna and Molliver, 1980).

The existence of γ-aminobutyric acid (GABA) in midbrain raphe nuclei has been repeatedly reported (Massari et al., 1976; Belin et al., 1979; Gamrani et al., 1979; Nanopoulos et al., 1982; Harandi et al., 1987). In fact, there is as much glutamate decarboxylase activity (a specific marker for GABAergic neurons; Fonnum and Walberg, 1973; Ribak et al., 1976) in DRN as in cerebellar GABA-rich nuclei (Massari et al., 1976). The failure to obtain important changes in γ-aminobutyric acid decarboxylase activity after lesioning afferents to the DRN, as well as the presence in this nucleus of terminals, dendrites, and nerve cell bodies accumulating [^{3}H]GABA (Belin et al., 1979), support the notion that the GABAergic network is predominantly intrinsic to the raphe. GABA-positive cell bodies are less numerous than those for 5-HT (Belin et al., 1979; Harandi et al., 1987), and in one of these studies, 40% of the GABA-positive somata was found to contain 5-HT, whereas 30% of the 5-HT-containing neurons immunostained for GABA (Harandi et al., 1987). Hence, it was proposed that some neurons could be both GABAergic and serotonergic. Coexistence of 5-HT and GABA in midbrain raphe neurons has been repeatedly reported (Nanopoulos et al., 1982; Gamrani et al., 1984; Harandi et al., 1987; Gao et al., 1993); however, the proportion and the nuclei in which colocalization occurs vary among different studies. For example, electron microscopy studies revealed coexistence of both neurotransmitters in the somata of DRN neurons (Harandi et al., 1987). On the other hand, confocal microscopy studies revealed that in DRN and MRN, 5-HT and GABAergic neurons constitute two largely distinct populations, double-labeled neurons being observed only within the raphe magnus, raphe obscurus, and raphe pallidus nuclei (Gao et al., 1993). The latter results have been confirmed by Stamp and Semba (1995) who also observed that in raphe sections only a very small percentage of 5-HT neurons in the medullary raphe nuclei also contain GABA (raphe magnus is the nucleus where the percentage of colocalization was highest and it reached only 3.6%). In the latter study, double-labeled cells were virtually absent in the midbrain raphe nuclei, constituting 0.1 to 0.7% of the total number of cells in DRN, MRN, and the supralemniscal region. Low occurrence of colocalization of 5-HT and GABA has also been found in cultured 5-HT neurons obtained from the ponto-mesencephalic region of neonatal rats (Johnson, 1994a). The use of postnatal rat midbrain-pontine 5-HT-neuron culture has also provided evidence for corelease, and hence colocalization, of 5-HT and glutamate from single 5-HT neurons (Johnson, 1994b). However, the major drawback of the latter technique is that the possibility of glutamatergic function having developed in culture may not be ruled out. Contrary to this last possibility is the fact that coexistence of 5-HT and glutamate in rat and primate brain has been observed in medullo-spinal pathways (Nicholas et al., 1992). 5-HT has also been found to colocalize with the trace amine tryptamine (Dabadie and Geffard, 1993) and with basic fibroblast nerve growth factor (bFGF) in DRN and MRN (Chadi et al., 1993). However, bFGF lacks the signal peptide necessary to be secreted by the classical exocytotic pathway used by neuropeptides (Mignatti et al., 1992) but is released when cells are injured. Rather than as a neurotransmitter, bFGF would then act as an autocrine and paracrine factor which elicits trophic responses. Indeed, a neurotrophic role has been postulated for 5-HT itself (for review, see Jacobs and Azmitia, 1992; Azmitia and Whitaker-Azmitia, 1995). Finally, colocalization of 5-HT and NADPH diaphorase in mesopontine neurons suggest that 5-HT neurons and nitric oxide may be used as neurotransmitters by the same neuron (Johnson and Ma, 1993; Wotherspoon et al., 1994).

C. Afferents to Midbrain 5-HT Nuclei

Afferent connections to the raphe nuclei have been studied using multiple techniques such as lesion and axon degeneration, histofluorescence, anterograde/retrograde tracer injection, autoradiography, and immunohistochemistry (Brodal et al., 1960; Fuxe, 1965; Aghajanian and Wang, 1977; Mosko et al., 1977; Sakai et al., 1977; Baraban and Aghajanian, 1981; Kalen et al., 1985; Stratford and Wirtshafter, 1988; Marzienkiewicz et al., 1989; Behzadi et al., 1990). Results from such studies have been summarized in Table 2.

Interconnections among raphe nuclei using retrograde-tracing techniques suggest a moderate-to-high density of 5-HT input. These observations contrast with those from most of the immunocytochemical or radioautographic ultrastructural studies indicating a low number of 5-HT fibers in the rostral raphe. Mosko et al. (1977) have reported that the main source of 5-HT fibers reaching the DRN arise either from the DRN itself or the

TABLE 1
Designation of serotonergic cell groups in the raphe nuclei and brainstem reticular formation and the corresponding classification into the B groups

Cytoarchitectonic Structure Containing 5-HT Neurons	B Group Classification
MRN, caudal part	B_5*
MRN, rostral main part	B_8*
DRN, caudal part	B_6
DRN principal, rostral part	B_7*
CLN	B_8
Nucleus pontis oralis	B_8/B_9*
Supralemniscal region	B_9

The structures marked * form part of the nucleus centralis superior as defined by Olszewski and Baxter in 1954. Table modified from Törk, 1990.

MRN. The latter input has been confirmed by other groups (Sakai et al., 1977; Aghajanian and Wang, 1977; Kalen et al., 1985; Vertes and Kocsis, 1994). However, given the proximity between injection and labeled sites, it cannot be ruled out that tissue damage and tracer diffusion may account for part of the retrograde staining observed in the MRN after tracer injection into the DRN. Furthermore, since retrograde transport of horseradish peroxidase by dendrites has been reported (Smith et al., 1974), this raises the possibility that the accumulation of tracer by dorsal and median raphe perikarya resulted from retrograde transport by dendrites, rather than the axon terminals of these neurons. On the other hand, tracer diffusion or retrograde dendrite transport cannot account for the projections arising from more distant nuclei (Sakai et al., 1977; Kalen et al., 1985; Marzienkiewicz et al., 1989; Behzadi et al., 1990). Histochemical confirmation of the neurotransmitter contained in connections among 5-HT nuclei has been performed on rare occasions (Stratford and Wirtshafter, 1988), and when done, numerous nonserotonergic cells were retrogradely labeled after tracer injection into the MRN. It is then possible that not all of the retrogradely labeled fibers are serotonergic. The general impression would be that 5-HT axons connecting 5-HT nuclei are "diluted" within the dorsal and median raphe neuropil.

An important afferent area to the raphe, both in terms of selectivity and density, is the lateral habenula (Aghajanian and Wang, 1977; Wang and Aghajanian, 1977b). This habenular circuit appears to be comprised of both a monosynaptic GABAergic pathway (Wang and Aghajanian, 1977b; Stern et al., 1981; Park, 1987) and a polysynaptic pathway in which GABA, Substance P (Neckers et al., 1979; Nishikawa and Scatton, 1985), and excitatory amino acids serve as components (Kalen et al., 1985, 1989).

Noradrenergic fibers impinge directly onto the dendrites of 5-HT neurons (Baraban and Aghajanian, 1981), producing an excitatory input on the firing activity of 5-HT neurons (Baraban and Aghajanian, 1980). The lateral hypothalamus also gives rise to a monosynaptic excitatory input to the DRN (Aghajanian et al., 1987) although the transmitter is unknown. Multiple afferent fibers immunoreactive for different neuropeptides, such as β-endorphin (Bloom et al., 1978), Substance P (Ljungdahl et al., 1978; Shults et al., 1984), neurotensin fibers (Uhl et al., 1979), CCK fibers (Vanderhaeghen et al., 1980), CLIP/adrenocorticotrophic hormone (ACTH) fibers (Romagnano and Joseph, 1983; Zheng et al., 1991; Léger et al., 1994), and VIP fibers (El Kafi et al., 1994), have also been described.

D. Efferent Pathways and Terminal Projection Areas

1. Efferent Pathways. The rostral 5-HT nuclei are the main source of 5-HT fibers projecting to telencephalon and diencephalon (Fuxe, 1965; Azmitia and Segal, 1978; Parent et al., 1981; Villar et al., 1987). Although the main contingent of fibers from these nuclei is ascending, they also innervate more sparely numerous brainstem structures (Vertes and Kocsis, 1994), the cerebellar cortex (Waterhouse et al., 1986; Zimny et al., 1988), and the spinal cord (Skagerberg and Björklund, 1985). In the nonhuman primate brain, two main ascending bundles have been described, a dorsal bundle (immediately ventral to the MLF), which receives fibers mainly from lateral wings and the ventromedial portion of the DRN, and a ventral bundle, which receives fibers from the midline DRN and MRN (Schofield and Everitt, 1981, Azmitia and Gannon, 1986). In the human fetus, two ascending axon bundles have also been observed, in the central gray, ventral to the fourth ventricle and the aqueduct ependyma, and between ventromedial and ventrolateral 5-HT groups (Nobin and Bjorklund, 1973). In the rat CNS, two major projection systems to the forebrain have also been described, a transtegmental system, which probably corresponds to the above-mentioned ventral bundle, courses through the midbrain forebrain bundle (MFB) and is the most prominent of the two systems described by Descarries in the rat brain, and a periventricular system, dorsally located along the longitudinal fasciculus of Schütz (Parent et al., 1981). In primates, unlike in rats, the dorsal component (dorsal raphe cortical tract) is much more developed than the MFB system, presumably due to an increase in fibers projecting to the cortex through the dorsal pathway. Moreover, the percentage of myelinated 5-HT fibers in rat MFB is 0.7% of the total immunoreactive 5-HT fibers, whereas it is as much as 25% in primate MFB (Azmitia and Gannon, 1983).

2. Terminal Projection Areas. Dorsal and median raphe nuclei each innervate specific terminal areas (Bobillier et al., 1975; Azmitia and Segal, 1978; Jacobs et al., 1978), and, in turn, each terminal projection area has its unique topographic representation within the respective nuclei. Labeling studies using wheat germ agglutinin, horseradish peroxidase, or fluorescent dyes have been used to unveil the midbrain raphe projection network. The more rostral portions of the midbrain raphe relate to the basal-ganglia-motor system and caudal areas are more related to the limbic system. Neurons projecting to the striatum occupy the caudal linear nucleus and a rostral portion of the DRN (Jacobs et al., 1978; Imai et al., 1986), whereas those projecting to substantia nigra (Imai et al., 1986) and the motor cortex (O'Hearn and Molliver, 1984; Waterhouse et al., 1986) reside within the rostral portions of the dorsal raphe (Imai et al., 1986). Hippocampus-projecting neurons are situated caudally in the DRN (caudal ventromedial portion and B_6), the MRN, and B_9 (Jacobs et al., 1978; Köhler and Steinbusch, 1982; Imai et al., 1986), similar to neurons projecting to the locus ceruleus (Imai et al., 1986) and to the entorhinal cortex (Köhler and Steinbusch, 1982). The raphe representation of the amygdala, on the other hand, bridges the "basal-ganglia-motor system" and the

TABLE 2
Afferents to the DRN

Structure of Origin	Projection Density	Neurotransmitter Implied	Reference
Telencephalic afferents			
Retina			Foote et al., 1978; Shen and Semba, 1994
Prefrontal cortex	Scarce	Possibly Ach	Arnsten and Goldman-Rakic, 1984
Subcortical forebrain afferents			Aghajanian and Wang, 1977
Diagonal band of brocca	Moderate-high	Possibly Ach	Aghajanian and Wang, 1977
	Low		Kalen et al., 1985
Stria terminalis	Scarce	AA	Aghajanian and Wang, 1977 Kalen et al., 1985
Diencephalic afferents			
Hypothalamic areas			
Lateral area	Moderate		Sakai et al., 1977; Berk and Filkenstein, 1982
	High	AA	Kalen et al., 1985
Dorsomedial area	High		Sakai et al., 1977
	Moderate	AA	Kalen et al., 1985
Parvocellular nucleus	Moderate-low		Kalen et al., 1985
Magnocellular nucleus	Moderate-low		Kalen et al., 1985
Perifornical area	Moderate		Sakai et al., 1977
	Moderate-low		Kalen et al., 1985
Preoptic area	Low		Aghajanian and Wang, 1977
	Moderate-low		Kalen et al., 1985
Arcuate nucleus	Low		Sakai et al., 1977; Saper et al., 1979
Submammillothalamic nucleus	Low		Kalen et al., 1985
Nucleus habenularis			
Lateralis Medialis	High	AA, GABA, Substance P	Aghajanian and Wang, 1977; Sakai et al., 1977; Herkenham and Nauta, 1977; Neckers et al., 1979; Stern et al., 1981; Kalen et al., 1985; Nishikawa and Scatton, 1985; Park, 1987; Kalen et al., 1989
Brainstem afferents			
Periaqueductal periventricular gray	Low	AA Neuropeptides	Kalen et al., 1985
Laterodorsal tegmental nucleus	Low		Sakai et al., 1977
VTA	Low-moderate	DA, AA	Aghajanian and Wang, 1977;
Sustantia nigra	Moderate-low		Sakai et al., 1977; Kalen et al., 1985
Locus ceruleus and subceruleus	High	NE	Roizen and Jacobowitz, 1976; Sakai et al., 1977; Grzanna and Molliver, 1980; Baraban and Aghajanian, 1981; Kalen et al., 1985
Nucleus tractus solitarius/A_2	Moderate	NE	Aghajanian and Wang, 1977; Sim and Joseph, 1989; Hebert and Saper, 1992
Paragigantocellular nucleus/C_1, C_2-C_3		Epinephrine	Sim and Joseph, 1989; Hebert and Saper, 1992
Parabrachial nuclei	Moderate-high		Sakai et al., 1977; Kalen et al., 1985
Raphe nuclei			
Caudalis linearis	Low	5-HT	Sakai et al., 1977
Dorsalis	Moderate	5-HT	Mosko et al., 1977
Medianus centralis superior	Moderate-high	5-HT EAA	Mosko et al., 1977; Sakai et al., 1977; Aghajanian and Wang, 1977; Kalen et al., 1985, 1988; Vertez and Martin, 1988
Magnus	Moderate-high	5-HT	Sakai et al., 1977; Kalen et al., 1985
Vestibular nuclei	Moderate-low		Sakai et al., 1977; Kalen et al., 1985
Prepositus hypoglossy	Moderate-low		Kalen et al., 1985
Nucleus principalis trigemini	Moderate-low		Kalen et al., 1985
Area dorsolateral to inferior olive	Moderate-low		Sakai et al., 1977; Kalen et al., 1985
Cortical afferents			
Prefrontal cortex	Moderate-high	Possibly Ach, AA	Aghajanian and Wang, 1977; Marzienkiewicz et al., 1989; Behzadi et al., 1990
Subcortical forebrain afferents			
Lateral septum	Scarce	AA	Marzienkiewicz et al., 1989; Behzadi et al., 1990
Diagonal band of brocca	Moderate-high Low	Possibly Ach Noncholinergic	Marzienkiewicz et al., 1989; Kalen and Wiklund, 1989; Behzadi et al., 1990
Stria terminalis	Scarce	AA	Marzienkiewicz et al., 1989; Behzadi et al., 1990
Amygdala	Moderate-low		Hopkins and Holstege, 1978; Marzienkiewicz et al., 1989; Behzadi et al., 1990
Nucleus accumbens	Low	AA	Marzienkiewicz et al., 1989; Behzadi et al., 1990
Ventral pallidum	Moderate		Behzadi et al., 1990

TABLE 2
Continued

Structure of Origin	Projection Density	Neurotransmitter Implied	Reference
Diencephalic afferents			
Hypothalamic areas			
Dorsomedial area	High	AA	Marzienkiewicz et al., 1989; Behzadi et al., 1990
Perifornical area	High	AA	Marzienkiewicz et al., 1989; Behzadi et al., 1990
Preoptic area	High	AA	Aghajanian and Wang, 1977; Villalobos and Ferssiwi, 1987; Marzienkiewicz et al., 1989; Behzadi et al., 1990
Arcuate nucleus Ventromedial nuclei Tuber cinereum	Low		Marzienkewicz et al., 1989; Behzadi et al., 1990
Premammillary, tuberomammillary, supramammillary, submammillothalamic nuclei	High		Marzienkewicz et al., 1989; Behzadi et al., 1990
Nucleus habenularis			
Lateralis	High	AA	Aghajanian and Wang, 1977; Herkenham and Nauta, 1977;
Medialis	Low		Marzienkiewicz et al., 1989; Behzadi et al., 1990
Brainstem afferents			
Periaqueductal Periventricular gray	Moderate-high	AA	Marzienkiewicz et al., 1989; Behzadi et al., 1990
Laterodorsal tegmental nucleus	High		Marzienkiewicz et al., 1989
VTA	Moderate-low	DA	Aghajanian and Wang, 1977;
Substantia nigra	Moderate-low		Marzienkiewicz et al., 1989; Behzadi et al., 1990
Interpeduncular nucleus	High	AA	Marzienkiewicz et al., 1989; Behzadi et al., 1990
Locus ceruleus and subceruleus	High	NE	Marzienkiewicz et al., 1989
Parabrachial nuclei	Moderate		Marzienkiewicz et al., 1989; Behzadi et al., 1990
Raphe nuclei			
Caudalis linearis	Low	5-HT	
Rostralis linearis	Moderate	5-HT	Marzienkiewicz et al., 1989
B_9	Moderate-high	5-HT/other	Stratford and Wirthshafter, 1988 Marzienkiewicz et al., 1989
Dorsalis	High	5-HT	Marzienkiewicz et al., 1989; Behzadi et al., 1990; Vertes and Kocsis, 1994
Pontis, magnus	Moderate	5-HT	Marzienkiewicz et al., 1989; Behzadi et al., 1990
Obscurus	Low	5-HT	Marzienkiewicz et al., 1989; Behzadi et al., 1990
Vestibular nuclei	Low		Marzienkiewicz et al., 1989
Motor nuclei: oculomotor, trochlear	Moderate-low	5-HT	Marzienkiewicz et al., 1989
Prepositus hypoglossy	Moderate-low		Marzienkiewicz et al., 1989; Behzadi et al., 1990

"limbic system representation" (Jacobs et al., 1978; Imai et al., 1986), possibly as a reflection of the functional diversity of amygdaloid nuclei. The consequence of this topographic arrangement is that the selective activation of a given functional group would simultaneously influence interconnected brain circuits. Neurons projecting to interrelated brain circuits, such as the sensorimotor cortex and cerebellar crus II, the visual cortex and cerebellar paraflocculus (Waterhouse et al., 1986), or to the substantia nigra and caudate-putamen (Imai et al., 1986), the entorhinal cortex and hippocampus (Köhler and Steinbusch, 1982), the trigeminal sensory complex and nucleus accumbens or amygdala (Li et al., 1993), arise from overlapping areas within the different nuclei. Moreover, in these regions, a single neuron may provide a common input to two different but functionally interrelated terminal areas. In the ventromedial portion of the DRN, the same neuron was found to project to at least three different forebrain structures related with the limbic system: septum, medial thalamus, and olfactory cortex (de Olmos and Heimer, 1980). Colateralization provides not only a means for producing concurrent influences on numerous functionally related circuits, but it is also a way of achieving extensive serotonergic innervation from a small number of raphe neurons.

In projection areas, serotonergic axons arising from DRN and MRN raphe have been distinguished on a morphological basis: in the rat brain, dorsal raphe fibers are extremely fine with minute varicosities (less than 1 μm in diameter), whereas those arising from median raphe are distinguished by large spherical varicosities (2–5 μm in diameter; Kosofsky and Molliver, 1987; Mulligan and Törk, 1988). In neocortical areas, dorsal raphe-fine axons have been found to be far more numerous than beaded axons (Kosofsky and Molliver, 1987; Mamounas et al., 1991) and to follow a rostrocaudal pattern of distribution with a greater concentration in more frontal regions. These findings are in agreement with those obtained in retrograde-labeling experiments in which the frontal cortex received twice as many projections from cells in the DRN than those in the parietal and occipital cortex (O'Hearn and Molliver, 1984). Mor-

phological duality has also been reported in the cat cortex (Mulligan and Törk, 1988), but such results were not replicated in the rat brain where immunostained varicosities exhibited similar shape and size irrespective of the cortical region or sector examined (Séguéla et al., 1989).

Over a wide range of doses (2.5–40 mg/kg) and survival times (1 week to 2 months), neurotoxic amphetamine derivatives, such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymetamphetamine (MDMA), and *p*-chloroamphetamine (PCA), produce degeneration of fine 5-HT axon terminals while sparing beaded 5-HT axons (Mamounas and Molliver, 1988; O'Hearn et al., 1988; Wilson et al., 1989; Mamounas et al., 1991), as well as raphe cell bodies (O'Hearn et al., 1988; Mamounas et al., 1991). The 5-HT-releasing agent fenfluramine produces similar effects (Molliver and Molliver, 1990). These neurotoxins have been used to determine the differential distribution of beaded and fine axons and hence the contribution of DRN and MRN in different projection areas. In the hippocampus and neocortex, different neurotoxins have been reported to produce a regional axon loss/sparing pattern which is coincident with fine and beaded axon distribution. *p*-chlorophenylalanine (PCPA) or MDA administration caused marked denervation in the parietal and occipital cortices, and a moderate number of axons was spared in the frontal cortex (Mamounas and Molliver, 1988; Mamounas et al., 1991). In the hippocampus, there was a greater density of spared axons, most probably due to the fact that in the hippocampal formation, in comparison to other cortical areas, beaded axons are especially prevalent (Mamounas et al., 1991). An exceptionally large number of axons was spared in stratum radiatum of CA_3, and intact axons were also found in the stratum oriens of CA_3, stratum lacunosum of CA_1, and flanking the granular layer in the dentate gyrus. In contrast, few 5-HT axons remained in the outer molecular layer of the dentate gyrus and stratum oriens and radiatum of CA_1, areas that normally receive DRN innervation (Mamounas et al., 1991). On the other hand, Oleskevich and Descarries (1990) described 5-HT axons projecting to the same layers, but no distinction between fine or beaded fibers was made. This same group has nevertheless reported that 5-HT varicosities with synaptic membrane specializations are slightly larger than their nonjunctional counterparts (Oleskevich et al., 1991). In fact, based on the observation that fine fibers rarely make synaptic contacts while larger varicosities found on beaded axons tend to show distinct synaptic specializations, Törk (1990) proposes that, along with dual morphology and distinct sensitivity to neurotoxins, the existence of axons with and without synaptic contacts supports the idea that the ascending raphe projections form a dual system. It should be noted that the reported percentages of synaptic incidence in terminal areas are very variable: 30 to 80% (of a total of 5.8×10^6 varicosities/mm^3) in the rat cortex (Papadopoulos et al., 1987; Séguéla et al., 1989), 3% in the monkey cortex (de Felipe and Jones, 1988), 10 to 15% (of a total of 2.6×10^6 varicosities/mm^3) in the rat neostriatum (Soghomonian et al., 1987), and 18 to 24% in the hippocampus (of a total of 2.7×10^6 varicosities/mm^3; Oleskevich et al., 1991).

III. Physiological and Pharmacological Aspects of the 5-HT System

A. Firing Activity of 5-HT Neurons

Midbrain raphe 5-HT neurons exhibit a spontaneous, slow (1–5 spikes/s), regular discharge pattern (Aghajanian and Vandermaelen, 1982; Vandermaelen and Aghajanian, 1983). Intracellular recordings from dorsal raphe neurons reveal that 5-HT cells undergo repetitive cycles of interspike hyperpolarization and depolarization, spikes arising from depolarizing ramps rather than from excitatory postsynaptic potentials (Aghajanian and Vandermaelen, 1982; Vandermaelen and Aghajanian, 1983). The ionic basis for this electrical activity is summarized in Fig. 1.

In freely moving cats, regular stereotyped intrinsic activity of 5-HT neurons remains unchanged over exposure to a hot environment or administration of a pyrogen, increase in blood pressure, insulin-induced hypo-

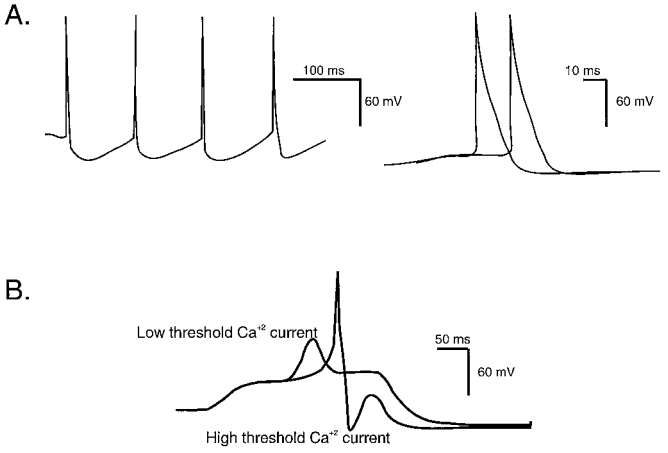

FIG. 1. Representative voltage tracings obtained from acutely isolated DRN neurons under current clamp (modified from Penington et al., 1991). A, spontaneous activity exhibited by the cell at resting membrane potential. Typical action potentials consist of an initiating ramp of depolarization, spike, shoulder upon repolarization, and an after-hyperpolarization. B, depolarization of a cell bathed with the Na^+ channel blocker TTX. TTX abolished the fast component of the action potential and uncovered low- and high-threshold Ca^{2+} components of the action potential. Although T channels seem to be responsible for the low-threshold current, at least three different channel types (including N- and L-type) underlie the high-threshold current (Penington and Kelly, 1990; Penington et al., 1991). The after-hyperpolarization that follows, as in many other vertebrae and invertebrae neurons, is mediated by a Ca^{2+}-activated K^+ outward current (Vandermaelen and Aghajanian, 1982; Aghajanian, 1985; Aghajanian et al., 1987). This after-hyperpolarization is responsible for a long-lasting refractory period and the slow firing rate of 5-HT neurons. As Ca^{2+} that entered during the action potential is sequestered/extruded, the Ca^{2+}-dependent K^+ current and the after-hyperpolarization decrease. When the membrane potential reaches again the value for the low-threshold Ca^{2+} current (approximately −60 mV), a new spike will be triggered. As repolarization from membrane potentials below the resting potential takes place, a voltage-dependent outward K^+ current that slows the rate of depolarization is simultaneously activated, the so-called I_A current (Aghajanian, 1985).

glycemia, administration of painful stimuli, physical restraint, or exposure to powerful aversive stimuli (see Jacobs and Fornal, 1993). However, the basic pattern of activity is not constantly the same, and it has been shown to change dramatically during the sleep-wake-arousal cycle. Firing activity progressively slows down from an aroused state through quiet waking and slow wave sleep (SWS), to become almost silent during rapid eye movement (REM) sleep (for review, see McGinty and Harper, 1976; Jacobs and Fornal, 1993). The suppression of firing of 5-HT neuron during REM sleep correlates well with the production of muscle atonia secondary to inhibition of motoneurons controlling antigravity muscles (Trulson et al., 1981; Steinfels et al., 1983). More recently, a relationship between motor output and 5-HT neuron activity has been observed. During quiet wakefulness, when cats engage in various types of stereotyped oral-buccal activities such as chewing and biting, licking, or grooming with the tongue, approximately 25% of DRN and MRN increase their firing activity 2- to 5-fold (see Jacobs and Azmitia, 1992; Jacobs and Fornal, 1993). This increased neuronal activity precedes the onset of stereotyped motor behaviors and ends with its offset; it does not occur during purposive episodic movements, but some of the neurons may be activated by somatosensory and proprioceptive stimulations of the head and neck area. These data have given support to the current motor hypothesis of 5-HT function in which the primary role of the 5-HT system would be facilitation of motor output and concurrent inhibition of sensory information processing (Jacobs and Fornal, 1993).

The firing activity of midbrain 5-HT neurons is controlled by two main mechanisms, i.e., autoregulatory influences arising from 5-HT neurons themselves and heteroregulation by local neurons or afferents to the raphe nuclei.

1. Autoregulation of 5-HT Neuron Firing Activity. The firing rate of 5-HT neurons is decreased by 5-HT, and this effect is mediated by somatodendritic 5-HT_{1A} autoreceptors (Aghajanian et al., 1972; Vandermaelen et al., 1986; Blier and de Montigny, 1987). 5-HT and 5-HT_{1A} agonists inhibit 5-HT firing activity by inducing membrane hyperpolarization which is brought about by a 2-fold mechanism, i.e., by increasing conductance to potassium ions (Aghajanian and Lakoski, 1984; Yoshimura and Higashi, 1985) and by reducing a high-threshold Ca^{2+} current (Fig. 1; Penington and Kelly, 1990; Penington and Fox, 1994). In both cases, the response to 5-HT is G protein-mediated via a direct interaction between G proteins and the respective ion channel (Innis and Aghajanian, 1987; Penington et al., 1991; Penington et al., 1993; Penington and Fox, 1994).

Current knowledge of the pharmacological properties of somatodendritic 5-HT_{1A} autoreceptors is mostly based on electrophysiological studies done in vivo which have assessed the effect of different compounds on the firing activity of 5-HT neurons. Table 3 summarizes these effects. Of the synthetic 5-HT_{1A} agonists listed therein, lysergic acid diethylamide (LSD) and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) are among the most effective in producing suppression of firing activity, not only of DRN 5-HT neurons but also that of $CA_{1/3}$ hippocampus pyramidal neurons. Unlike 8-OH-DPAT which produces maximal activation of postsynaptic 5-HT_{1A} receptors, most of the drugs that completely inhibit 5-HT neuron firing activity produce partial agonistic effect at postsynaptic 5-HT_{1A} sites in CA_1 and CA_3 regions of the hippocampus (Yocca and Maayani, 1985; Yocca et al., 1986; Andrade and Nicoll, 1987a). Some of these compounds (e.g., tandospirone and flesinoxan) not only produce a smaller maximal response than 8-OH-DPAT but have also been shown to reduce the inhibitory effect induced by the microiontophoretic application of 5-HT onto CA_3 pyramidal neurons (Godbout et al., 1991; Hadrava et al., 1995). Conversely, several compounds previously thought to act as 5-HT_{1A} receptor antagonists at postsynaptic sites have been shown to produce submaximal agonistic effects at presynaptic sites, inhibiting 5-HT neuron firing. These compounds are also best classified as partial agonists. For example, drugs like (+)-WAY 100135, NAN-190, or BMY 7378 block the inhibition induced by 5-HT_{1A} agonists on forskolin-stimulated adenylyl cyclase activity from hippocampal membranes (Rydelek-Fitzgerald et al., 1990), block the postsynaptic neurochemical, electrophysiological, and behavioral effects of 8-OH-DPAT (Chaput and de Montigny, 1988; Sharp et al., 1990; Routledge et al., 1993; Escandon et al., 1994), but they also decrease 5-HT neuron firing activity (Vandermaelen et al., 1987; Chaput and de Montigny, 1988; Fletcher et al., 1993, Haddjeri and Blier, 1995). The suppression of raphe firing caused by NAN-190 and BMY 7378 is blocked by the β-adrenergic/5-HT_{1A} antagonist (±)-propranolol (Middlemiss, 1984a; Tricklebank et al., 1985; Greuel and Glaser, 1992). Similarly, the inhibition induced by SDZ 216-235 on 5-HT neuron firing is blocked by the β-adrenergic/5-HT_{1A} antagonist (−)-tertatolol (Lanfumey et al., 1993; Lejeune et al., 1993), further indicating that these "postsynaptic antagonists" act as partial agonists on presynaptic 5-HT_{1A} receptors. The suppression of 5-HT neuron firing activity induced by (+)-WAY 100135 may be overcome by pretreatment with the α_1-adrenergic agonist phenylephrine but not by (−)-tertatolol, suggesting that the latter effect is not due to partial agonism on 5-HT_{1A} receptors, but rather to the blockade of firing stimulating α_1 adrenoceptors on 5-HT neurons (Lanfumey et al., 1993). Because WAY 100635 and (−)-tertatolol have been consistently shown to block the effect of 5-HT_{1A} agonists without affecting 5-HT neuron firing (Jolas et al., 1993; Lanfumey et al., 1993; Lejeune et al., 1993; Prisco et al., 1993; Craven and Grahame-Smith, 1994; Fletcher et al., 1994; Mundey et al., 1994; Table 3), they have been named "silent" antagonists. In spite of its lack of partial agonistic activity on somatodendritic 5-HT_{1A}

TABLE 3
Effect of 5-HT$_{1A}$ active drugs on 5-HT neuron firing activity

Drug	Effect on 5-HT Neuron Firing	Dose	Reference
In vitro studies			
(−)-Propranolol	◀▶[a]	10 μM	Kidd et al., 1993
(±)-Tertatolol	◀▶	1 μM	Kidd et al., 1993
(−)-Tertatolol	◀▶	10 nM–10 μM	Jolas et al., 1993
WAY 100635	◀▶	30 nM	Craven and Grahame-Smith, 1994
(+)-WAY 100135	◀▶	10 nM–1 μM	Lanfumey et al., 1993
In vivo studies			
(−)-Alprenolol	◀▶	>2 mg/kg i.v.	Millan et al., 1994
Spiperone	◀▶	1 mg/kg i.v.	Lum and Piercey, 1988; Blier et al., 1989b, 1993
S-UH-301	◀▶▲	<0.25 mg/kg i.v.	Arborelius et al., 1995
(−)-Tertatolol	◀▶	0.5–4 mg/kg i.v.	Lejeune et al., 1993
(−)-Tertatolol	◀▶	>2 mg/kg i.v.	Millan et al., 1994
(−)-Tertatolol	◀▶	0.1–0.5 mg/kg i.v.	Jolas et al., 1993
(−)-Tertatolol	◀▶	1 mg/kg i.v.	Prisco et al., 1993
(−)-Tertatolol	◀▶	ID_{50} > 4 mg/kg i.v.	Gobert et al., 1995
(+)-WAY 100135	◀▶	0.1–1 mg/kg i.v.	Lejeune et al., 1993
(+)-WAY 100135	◀▶	0.5 mg/kg i.v.	Piñeyro et al., 1996
(+)-WAY 100635	◀▶▲	0.1–0.5 mg/kg i.v.	Fornal et al., 1994
(+)-WAY 100135	◀▶	1 mg/kg i.v.	Haddjeri and Blier, 1995
In vitro studies			
8-OH-DPAT	▼	3 nM–1 μM	Lanfumey et al., 1993
8-OH-DPAT	▼	IC_{50}, 10 nM	Jolas et al., 1993
8-OH-DPAT	▼	IC_{50}, 11 nM	Schechter et al., 1990
CM 57493	▼	5–20 μM	Adrien et al., 1989
Ipsapirone	▼	50 nM	Jolas et al., 1993
Ipsapirone	▼	60 nM	Schechter et al., 1990
Lesopitron	▼	300 nM	Jolas et al., 1993
(+)-20499	▼	IC_{50}, 6 nM	Kidd et al., 1993
(−)-S20500	▼	IC_{50}, 130 nM	Schechter et al., 1990
BMY 7378	▼	100–150 nM	Vandermaelen et al., 1987
BMY 7378	▼	IC_{50}, 10 nM	Greuel and Glaser, 1991
NAN-190	▼	IC_{50}, 20 nM	Greuel and Glaser, 1991
SDZ 216525	▼	100 nM	Hamon et al., 1993
SDZ 216525	▼	100 nM–1 μM	Lanfumey et al., 1990
(+)-WAY 100135	▼	1 μM	Hamon et al., 1993
(+)-WAY 100135	▼	>1 μM	Lanfumey et al., 1993
In vivo studies			
BAY X 3702	▼	0.45 μg/kg i.v.	Dong et al., 1998
8-OH-DPAT	▼	2 μg/kg i.v.	Blier and de Montigny, 1987; Blier et al., 1989b
8-OH-DPAT	▼	1.5 μg/kg i.v.	Fornal et al., 1994a
8-OH-DPAT	▼	1.5 μg/kg i.v.	Prisco et al., 1993
8-OH-DPAT	▼	2.2 μg/kg i.v.	Jolas et al., 1994
8-OH-DPAT	▼	0.6 μg/kg i.v.	Gobert et al., 1995
8-OH-DPAT	▼	1.6 μg/kg i.v.	McCall et al., 1994
8-OH-DPAT	▼	0.4 μg/kg i.v.	Arborelius et al., 1994
8-OH-DPAT	▼	1.5 μg/kg i.v.	Cox et al., 1990
5-MeOT	▼	45 μg/kg i.v.	Fornal et al., 1994a
Buspirone	▼	75 μg/kg i.v.	Fornal et al., 1994a
Buspirone	▼	15 μg/kg i.v.	McCall et al., 1994
CM 57493	▼	5–20 mg/kg i.p.	Adrien et al., 1989
Flesinoxan	▼	108 μg/kg i.v.	Hadrava et al., 1995
Flesinoxan	▼	21 μg/kg i.v.	Gobert et al., 1995
Gepirone	▼	10 μg/kg i.v.	Blier and de Montigny, 1987
Ipsapirone	▼	83 μg/kg i.v.	Fornal et al., 1994
Ipsapirone	▼	7.5 μg/kg i.v.	Jolas et al., 1994
Ipsapirone	▼	3.7 μg/kg i.v.	Gobert et al., 1995
Ipsapirone	▼	30 μg/kg i.v.	Cox et al., 1990
Ipsapirone	▼	0.1–1 mg/kg i.p.	Adrien et al., 1993
LSD	▼	5 μg/kg i.v.	Blier et al., 1991; Godbout et al., 1991
LY 293284	▼	0.08 μg/kg s.c.	Foreman et al., 1994
S 14671	▼	0.16 μg/kg i.v.	Gobert et al., 1995

TABLE 3
Continued

Drug	Effect on 5-HT Neuron Firing	Dose	Reference
S 14506	↓	0.3 μg/kg i.v.	Gobert et al., 1995
S 14671	↓	0.16 μg/kg i.v.	Lejeune et al., 1993
S 14489	↓	5 μg/kg i.v.	Millan et al., 1994
S 15535	↓	7 μg/kg i.v.	Millan et al., 1994
S 15931	↓	30 μg/kg i.p.	Millan et al., 1994
SR 57746A	↓	90–250 μg/kg i.v.	Bachy et al., 1993
Tandospirone	↓	3.4 μg/kg i.v.	Gobert et al., 1995
Tandospirone	↓	9.1 μg/kg i.v.	Godbout et al., 1991
U 92016A	↓	3.4 μg/kg i.v.	McCall et al., 1994
WY 48723	↓	0.6 μg/kg i.v.	Gobert et al., 1995
Zalospirone	↓	3.6 μg/kg i.v.	McCall et al., 1994
BMY 7378	↓	17.5 μg/kg i.v.	Cox et al., 1990
BMY 7378	↓	1.5 μg/kg i.v.	Millan et al., 1994
BMY 7378	↓	1.6 μg/kg i.v.	Gobert et al., 1995
BMY 7378	↓	15.3 μg/kg i.v.	Fornal et al., 1994
MDL73005EF	↓	4 μg/kg i.v.	Millan et al., 1994
PAPP (LY165163)	↓	42 μg/kg i.v.	Sprouse and Aghajanian, 1987
Flibanserin	↓	239 μg/kg i.v.	Rueter et al., 1998
In vivo studies			
MDL 73005EF	↓	4.4 μg/kg i.v.	Gobert et al., 1995
NAN-190	↓	4 μg/kg i.v.	Millan et al., 1994
NAN-190	↓	3.9 μg/kg i.v.	Gobert et al., 1995
NAN-190	↓	34.2 μg/kg i.v.	Fornal et al., 1994b
SDZ 216525	↓	0.1–0.5 mg/kg i.v.	Mundey et al., 1994
SDZ 216525	↓	0.4 mg/kg i.v.	Fornal et al., 1994
SDZ 216525	↓	47.3 μg/kg i.v.	Gobert et al., 1995
S-UH-301	↓	0.5–4 mg/kg i.v.	Arborelius et al., 1994
(+)-WAY 100135	↓	0.4 mg/kg i.v.	Fletcher et al., 1994
(+)-WAY 100135	↓	0.25–0.50 mg/kg i.p.	Arborelius et al., 1995
(+)-WAY 100135	↓	31 μg/kg i.v.	Escandon et al., 1994
(+)-WAY 100135	↓	0.1–1 mg/kg i.v.	Fornal et al., 1994

Note: drugs have been ordered according to their efficacy in different systems. Because efficacy is system-dependent, drugs that figure as partial agonists in one experimental paradigm may appear as antagonists in another.
[a] ↔, no effect.

receptors, (+)-WAY 100135 does not enter into this category due to its α_1-adrenergic blocking properties (Lanfumey et al., 1993) which induce an observable inhibition of firing in the electrophysiological paradigm.

Two classical hypotheses have been proposed to explain the distinct pharmacological properties of pre- and postsynaptic 5-HT_{1A} receptors: 1) greater receptor reserve in somatodendritic than in postsynaptic areas (Meller et al., 1990; Yocca, 1990; Greuel and Glaser, 1992; Millan et al., 1992) and 2) different pre- and postsynaptic receptors (see de Montigny and Blier, 1992a,b). The first hypothesis sustains that if no receptor reserve exists, like in the case of the hippocampus (Yocca, 1990; Gozlan et al., 1994), partial agonists will not produce a maximal effect and may block the effect of full agonists. Conversely, if spare receptors are present, at an appropriately high dose, drugs with low intrinsic activity (i.e., partial agonists) may still elicit a maximal response and appear as full agonists. The second hypothesis, suggesting distinct 5-HT_{1A} receptors at pre- and postsynaptic sites has been proposed to account not only for the differential pre-/postsynaptic activity of partial agonists but also to explain the following observations: 1) different rank order in the effectiveness of 5-HT_{1A} agonists to inhibit DRN (8-OH-DPAT, gepirone > LSD > 5-HT) versus hippocampus firing activity (5-HT > gepirone > 8-OH-DPAT; Blier and de Montigny, 1987, 1990; Chaput and de Montigny, 1988); 2) preferential antagonistic effect of BMY 7378 at postsynaptic sites (Chaput and de Montigny, 1988) and of spiperone at presynaptic receptors (Lum and Piercey, 1988; Blier et al., 1989b, 1993a; Fornal et al., 1994a); and 3) differential effect of long-term administration of 5-HT_{1A} agonists which induce desensitization of presynaptic but not of postsynaptic CA_3 5-HT_{1A}-mediated responses (Blier and de Montigny, 1994).

Over the past few years advances in molecular biology, i.e., the expression of G protein-coupled receptors (GPCRs) in surrogate cell systems, have greatly modified the way in which we understand pharmacological properties of GPCRs and consequently the mechanistic drug-receptor models used to simulate drug action (see Kenakin, 1996). The first of such models to consider that R activation by an agonist would result in R-G protein

coupling, and subsequent activation of the G protein was the ternary complex model (De Léan et al., 1980). This model has been the standard by which drug-receptor interactions have been explained in the past. More recently, the observed ability of GPCRs for becoming activated independent of the presence of an agonist (Samama et al., 1993; Barker et al., 1994; Chidiac et al., 1994) has guaranteed the appearance of alternative paradigms (extended ternary complex model, Samama et al., 1993; two-state model, Leff, 1995; cubic ternary complex model, Weiss et al., 1996). All models consider that agonist-independent activity is secondary to spontaneous isomerization between the inactive (R) and active (R^*) states of the receptor. Furthermore, according to all of these models, agonists have preferential affinity and stabilize the active conformation of the receptor (R^*). Ligands that stabilize the resting state (R), decreasing the number of spontaneously active receptors, act as inverse agonists, and antagonists would be those drugs that present similar affinities for both, favoring neither state of the receptor. The fundamental consequence of embracing any such paradigms is that affinity, potency, and/or efficacy values should be considered receptor- and system-dependent, the basal $R:R^*$ ratio being a determinant of this dependence. Antagonists, whose effect would remain unmodified by the level of spontaneous activation, would therefore remain as the most reliable tools for receptor classification.

Because none of the drugs producing differential pre- and postsynaptic effects are neutral antagonists (note: spiperone has been recently characterized as an inverse agonist on spontaneously active human 5-HT_{1A} receptors expressed in Chinese hamster ovary cells; Newman-Tancredi et al., 1997), it cannot be ruled out that differences in efficacy observed in DRN and hippocampus are not due to $R:R^*$ ratios. Furthermore, differential long-term desensitization of presynaptic receptors following long-term administration of 5-HT_{1A} agonists has been attributed to differential drug efficacy in midbrain and hippocampus (see de Montigny and Blier, 1992a,b). Hence, possible different $R:R^*$ ratios could also account for the latter observation. Finally, differences in the $R:R^*$ ratio may explain a set of drugs being more efficacious in one area than in another, but it does not account for the observation that rank order of agonist efficacy may be different in two regions. The observation of different rank order of efficacy for 5-HT, gepirone, and 8-OH-DPAT in DRN and hippocampus could be due either to the presence of different 5-HT_{1A} receptor subtypes or to the same 5-HT_{1A} receptor protein coupling to different G proteins in the two different regions (see Kenakin, 1995 for receptor promiscuity). Paradigms which do not include G proteins in their formulation, such as the two-state model (Leff, 1995), do not account for the latter possibility. A more recent version, the three-state model (Leff et al., 1997), which allows for a single receptor to couple with two different G protein pathways, may be invoked to explain the differences in pharmacology in pre- and postsynaptic 5-HT_{1A} receptors without the need to propose different receptor subtypes. The coupling of a single receptor to more than one G protein pathway is also addressed by the cubic ternary model. The latter paradigm has the advantage of providing yet another mechanism to explain changes in rank order of efficacy of agonists. The mechanism, known as agonist trafficking of receptor signals (Kenakin, 1995), proposes that different drugs may provoke agonist-specific receptor conformations which would selectively promote G protein coupling in response to activation by different agonists. The possibility of a given conformation being more easily favored in one structure than in another may explain the changes in agonist rank order. Furthermore, the fact that i.c.v. administration of cholera toxin does not alter responsiveness of 5-HT neurons to microiontophoretic applications of 5-HT or 8-OH-DPAT, but reduces that of CA_3 neurons by 90% (Blier et al., 1993b), may also be explained by differential G protein coupling in the two regions.

Extracellular availability of endogenous 5-HT at the cell body level and tonic activation of somatodendritic 5-HT_{1A} autoreceptors is yet another factor that may contribute to modify the effects of 5-HT_{1A} ligands. Thus, Fornal et al. (1994a) have shown that the acute i.v. administration of the 5-HT_{1A} agonist 8-OH-DPAT or of the partial agonists ipsapirone and buspirone was more effective in inhibiting 5-HT neuron firing activity when cats were inactive (drowsiness) than during active wakefulness (a period of higher neuronal activity, 5-HT neuron depolarization, and hence of 5-HT release). Conversely, the antagonists spiperone and WAY 100635 induced a dose-dependent increase in the firing rate of 5-HT neurons, which was evident during wakefulness but not during sleep (Fornal et al., 1994a,c) when 5-HT neurons are silent, and therefore there is very low depolarization-mediated release of 5-HT to activate somatodendritic 5-HT_{1A} receptors. Moreover, in anesthetized rats and guinea pigs, in which 5-HT neuron firing activity resembles that of SWS, WAY 100635 had no effect on dorsal raphe neuronal firing (Fletcher et al., 1994; Mundey et al., 1994). It is also worth noting that the 5-HT_{1A} antagonistic properties of (+)-WAY 100135 on spontaneous 5-HT neuron firing were not evident in anesthetized rats (Haddjeri and Blier, 1995), in awake freely moving cats (Fornal et al., 1994c), or in an antidromic stimulation paradigm in which 5-HT neuron firing is suppressed by somatodendritic release of 5-HT (Piñeyro et al., 1996b). Yet, at doses similar to the ones used in previous studies (0.5–1 mg/kg i.v.), (+)-WAY 100135 reversed the suppression of 5-HT neuron firing activity induced by the blockade of 5-HT uptake (Arborelius et al., 1995; Hajós et al., 1995). This observation is in keeping with the previous assumption that the action of 5-HT_{1A} ligands on 5-HT neuron firing activity may

differ according to the tonic activation of their somatodendritic autoreceptors by the extracellular concentration of 5-HT. Similarly, S-UH-301, which in the dose range of 0.5 to 4 mg/kg i.v. reduces 5-HT neuron firing, effectively blocked the suppressant effect induced by the systemic administration of selective 5-HT reuptake blocker citalopram (Arborelius et al., 1994, 1995). Moreover, pindolol, which by itself induces no change in 5-HT neuron firing activity at low doses, prevents the inhibitory effect of paroxetine and LSD on this parameter (Romero et al., 1996; Haddjeri et al., 1999).

Pindolol is an antagonist which, in the electrophysiological paradigm, discriminates between presynaptic and postsynaptic hippocampal 5-HT_{1A} receptors. Like spiperone, pindolol blocks the effect of endogenous 5-HT on presynaptic receptors without interfering with the inhibitory effect induced on CA_3 pyramidal neuron firing activity by the microiontophoretic application of 5-HT (Blier et al., 1993a; Romero et al., 1996). Moreover, pindolol, in contrast with other 5-HT_{1A} antagonists, was recently shown to be ineffective in blocking the inhibitory effect of 5-HT_{1A} agonists on the firing activity of CA_1 pyramidal neurons in freely moving rats (Tada et al., 1999). These data do not imply that under certain experimental conditions pindolol cannot act as a 5-HT_{1A} antagonist, as shown in the in vitro studies of Corradetti et al. (1998) carried out on hippocampus slices. WAY 100635, on the other hand, has been shown to block the effect of 5-HT on pre- and postsynaptic 5-HT_{1A} receptors located on CA_3 pyramidal neurons (Haddjeri et al., 1996). Binding studies are consistent with the latter observation, i.e., [^{3}H]WAY 100635 behaves as an antagonist in projection areas such as the hippocampus since guanyl nucleotides fail to influence its K_D and B_{max} parameters (Khawaja, 1995; Khawaja et al., 1995). The presynaptic selectivity of the other silent antagonist (−)-tertatolol remains to be determined, and, for the time being, pindolol seems the only useful alternative of a presynaptic 5-HT_{1A} antagonist to be used in clinical trials. Finally, it is also worth mentioning the case of (−)-propranolol, which in spite of its moderate affinity for 5-HT_{1A} receptors (Pazos and Palacios, 1985; Hoyer and Schoeffter, 1991; Prisco et al., 1993), unlike pindolol, does not block the inhibitory effect of 5-HT on 5-HT neuron firing activity (Sprouse and Aghajanian, 1986; Blier et al., 1989a). Moreover, propranolol does not block the effects of either 5-HT or 8-OH-DPAT on hippocampal neuron firing activity. However, propranolol has been shown to block 5-HT-induced inhibition of forskolin-stimulated adenyl cyclase in hippocampal cells (Dumuis et al., 1988). These differences could be reconciled by assuming that the propranolol-bound-receptor conformation does not interfere with the G_i/K^+ channel pathway but blocks that of G_i/adenylate cyclase.

The local somatodendritic autoreceptor "short-loop" is only one of the autoregulatory mechanisms of 5-HT neuron firing activity. Several observations suggest that, in fact, the systemic administration of 8-OH-DPAT may regulate 5-HT neuron firing activity by an alternative "long feedback loop", which also entails 5-HT_{1A} receptor activation: 1) the effectiveness of microiontophoretic application of 5-HT_{1A} agonists to inhibit 5-HT neuron firing activity, but not that of systemic 8-OH-DPAT administration, is reduced following long-term treatment with the 5-HT_{1A} agonist gepirone and the selective serotonin reuptake inhibitor (SSRI) cericlamine (Blier and de Montigny, 1987; Jolas et al., 1994); 2) the effect of i.v. 8-OH-DPAT on 5-HT neuron firing activity, but not that of its microiontophoretic application onto 5-HT neurons, is increased by short-term lithium treatment (Blier et al., 1987); 3) the inhibitory effect of i.v. administration of 8-OH-DPAT on the firing activity of dorsal raphe serotonergic neurons in rats is attenuated by lesion of the frontal cortex (Ceci et al., 1994); and 4) the local application of pertussis toxin into the dorsal raphe (which inactivates $G_{i/o}$-coupled 5-HT_{1A} autoreceptors; Innis and Aghajanian, 1987) reduces the effectiveness of intraraphe, but not of systemic 8-OH-DPAT administration, to reduce 5-HT release in terminal projection areas (Romero et al., 1994). Direct evidence for such a long feedback loop being involved in mediating the activity of 5-HT neurons has recently been provided in electrophysiological and microdialysis experiments. The local application of 5-HT_{1A} agonists in the medial prefrontal cortex produces both a suppression of firing of 5-HT neurons and a decrease in extracellular synaptic level of 5-HT in the dorsal raphe, most likely as a result of the former phenomenon (Hajós and Sharp, 1998; Artigas et al., 1998).

2. Role of Non-5-HT Receptors in the Regulation of 5-HT Neuron Firing Activity. Autoradiographic and binding studies have documented the presence of different 5-HT-binding sites in the rat raphe nuclei (Waeber et al., 1988; Herrick-Davis and Titeler, 1988; Waeber et al., 1989a; Laporte et al., 1992). However, the role of 5-HT receptors, other than 5-HT_{1A} in modulating 5-HT neuron firing activity at the cell body level, has not been confirmed. The lack of effect of 1-[3-(trifluoromethyl)-phenylpiperazine (TFMPP) and *m*-chlorophenylpiperazine on the firing activity of 5-HT neurons led Sprouse and Aghajanian (1986, 1987) to conclude that 5-HT_{1B} receptors were not involved in regulating 5-HT neuron firing activity in the rat brain. The systemic injection and the microiontophoretic application of the preferential 5-HT_{2A} agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) reduce 5-HT neuron firing frequency. However, this effect could not be blocked by the 5-$HT_{2A/2C}$ antagonists ketanserin and ritanserin (Wright et al., 1990; Garratt et al., 1991). Moreover, the microiontophoretic application of ketanserin does not block the inhibition of 5-HT neuron firing induced by the neurotransmitter itself. It does, however, reduce the basal firing rates in the majority of 5-HT cells tested (Lakoski and Aghajanian, 1985). These observations, along with

the fact that the effectiveness of systemic 8-OH-DPAT but not that of DOI to inhibit the firing activity of 5-HT neurons is decreased following repeated DOI administration (Kidd et al., 1991), suggest that 5-HT_2 receptors are not directly involved in the regulation of 5-HT neuron firing. The role of 5-HT_3 receptors in regulating 5-HT neuron firing activity has also been investigated. The fact that systemic administration of the 5-HT_3 antagonist BRL 46470A does not block the reduction of the dorsal raphe 5-HT neuron firing rate induced by the microiontophoretic application of the 5-HT_3 agonist 2-methyl-5-HT (Haddjeri and Blier, 1995), and that 5-HT neuron firing activity remains unchanged after systemic administration of three different 5-HT_3 receptor antagonists, MDL 72222, ICS-205-930, and ondansetron (Adrien et al., 1992), indicate that 5-HT_3 receptors do not contribute to the regulation of 5-HT neuron firing activity. 8-OH-DPAT has often been used as the "gold standard" for defining 5-HT_{1A} receptors, but there is evidence that it also has reasonable good affinity at the rat (Shen et al., 1993) and guinea pig (To et al., 1995) 5-HT_7 receptor. Moreover, 8-OH-DPAT has also been identified as the most potent agonist at a cyclase-linked receptor not yet fully characterized (Becker et al., 1992), and it also binds to the serotonin transporter (SERT) (Schoemaker and Langer, 1988; Alexander and Wood, 1988; Ieni and Meyerson, 1988). However, the fact that methiothepin blocks 5-HT_7-mediated responses (Terron, 1997; Kitazawa et al., 1998) but not the effect of i.v. 8-OH-DPAT (Blier et al., 1989b) suggests that 5-HT_7 receptors are not involved in 8-OH-DPAT actions on 5-HT neuron firing activity. Furthermore, the dose at which 8-OH-DPAT interferes with 5-HT uptake is two to three orders of magnitude higher than the one currently used in electrophysiological experiments to assess drug effect on neuronal firing activity. Also, without excluding the role of other 5-HT receptors, the extensive list of 5-HT_{1A} compounds in Table 3 confirms the role of the latter receptor in regulating 5-HT neuron firing.

3. Heteroregulation of 5-HT Neuron Firing Activity. *N*-methyl-D-aspartate (NMDA) receptors may elicit excitatory postsynaptic potentials (EPSPs) (Pan and Williams 1989; Pinnock, 1992; Johnson, 1994a) and increase the firing activity of 5-HT neurons (Alojado et al., 1994) in vitro, but they do not seem to maintain a tonic activation of 5-HT neuron firing in vivo (Levine and Jacobs, 1992). However, glutamate does mediate the increase in firing activity observed following presentation of phasic auditory stimuli (Levine and Jacobs, 1992). In turn, excitatory amino acid (EAA) release in the dorsal raphe is negatively regulated by κ-opioid receptors (Pinnock, 1992). Rather unexpectedly, the systemic administration of the NMDA channel blocker (+)-MK-801 has been shown to facilitate the electrical activity of 5-HT neurons in the DRN. Such an observation could be explained by assuming that the channel blocker reduces the facilitation of an inhibitory influence. Microiontophoretic application of GABA onto dorsal raphe 5-HT neurons produces an inhibition of their firing rate (Gallager and Aghajanian, 1976; Levine and Jacobs, 1992). Also, the GABA blocker picrotoxin reduces the suppressant effect on 5-HT neuron activity caused by habenula and pontine reticular formation stimulation (Wang et al., 1976; Wang and Aghajanian, 1977; Stern et al., 1981). In freely moving animals, microiontophoretic application of bicuculline produces a significant increase of 5-HT neuron firing activity during SWS, but not during REM or quiet waking, indicating that GABAergic input is state-dependent and not tonic (Levine and Jacobs, 1992). In anesthetized rats, microiontophoretic and systemic administration of α adrenoceptor antagonists as well as 6-hydroxydopamine pretreatment suppress 5-HT neuron firing activity, suggesting a tonic facilitatory role for noradrenaline on dorsal raphe 5-HT neurons (Baraban and Aghajanian, 1980). Furthermore, activation of α_2-adrenergic autoreceptors by clonidine decreases norepinephrine (NE) output and suppresses the firing activity of 5-HT neurons (Clement et al., 1992; Haddjeri et al., 1996a). In contrast, systemic administration of α adrenoceptor antagonists in the awake freely moving animals produced no change in 5-HT neuron firing activity (Heym et al., 1981). Unlike EAAs, NE does not elicit EPSPs on 5-HT neurons, rather it suppresses the voltage-dependent K^+ current I_A (Fig. 1; Aghajanian, 1985), leading to a more rapid activation of the low-threshold inward calcium current that triggers the spike at the end of the pacemaker cycle of these neurons. Activation of CCK_A receptors located on 5-HT neurons also stimulates their firing activity (Boden et al., 1991), as does bombesin via the stimulation of neuromedin B receptors (Pinnock et al., 1994).

B. Effect of Antidepressant Drug Administration on 5-HT Neuron Firing Activity

The firing activity of 5-HT neurons is not inhibited only by administration of 5-HT_{1A} agonists (Vandermaelen et al., 1986; Blier and de Montigny, 1987; Godbout et al., 1990; Schechter et al., 1990; Hadrava et al., 1995). This effect is also produced by drugs such as SSRIs (Blier and de Montigny, 1983; Blier et al., 1984; Chaput et al., 1986b; Jolas et al., 1994; Arborelius et al., 1995; Hajós et al., 1995) and monoamine oxidase inhibitors (MAOIs) (Blier and de Montigny, 1985; Blier et al., 1986a,b), which induce an activation of 5-HT_{1A} autoreceptors due to an immediate increase in extracellular 5-HT at the somatodendritic level (see Sharp and Hjorth, 1990; Artigas, 1993). However, following sustained administration of SSRIs, MAOIs, and 5-HT_{1A} agonists, a progressive desensitization of somatodendritic 5-HT_{1A} autoreceptors takes place, and the effectiveness of 5-HT_{1A} agonists and antidepressant drugs to inhibit 5-HT neuron firing activity decreases. After 14 to 21 days of treatment, 5-HT neurons recover their pre-

treatment firing frequency (Blier and de Montigny, 1983, 1985, 1987; Blier et al., 1984, 1986; Chaput et al., 1986b; Chaput et al., 1991; Godbout et al., 1990; Schechter et al., 1990; Jolas et al., 1994; Arborelius et al., 1995; Hadrava et al., 1995; Dong et al., 1997, 1998). These multiple in vivo and in vitro electrophysiological studies have suggested that desensitization of somatodendritic 5-HT_{1A} autoreceptors could be a possible explanation for the recovery of 5-HT neuron firing activity. However, despite the fact that considerable functional evidence supports the occurrence of 5-HT_{1A} autoreceptor desensitization, the mechanism underlying this adaptative process remains unclear. The possibility that down-regulation of 5-HT_{1A} receptors in the midbrain may mediate the observed electrophysiological desensitization has not been confirmed. Although long-term treatment with gepirone (Welner et al., 1989), buspirone (Gobbi et al., 1991), and fluoxetine (Li et al., 1994) reduce the total number of [^{3}H]8-OH-DPAT-binding sites in midbrain raphe nuclei, sustained administration of citalopram, sertraline (Hensler et al., 1991), paroxetine, fluoxetine (Le Poul et al., 1995), and ericlamine (Jolas et al., 1994), as well as that of clorgyline, phenelzine, tranylcypromine (Hensler et al., 1991), and ipsapirone (Schechter et al., 1990), did not modify [^{3}H]8-OH-DPAT-binding parameters in the same region (all drugs were administered for a 14- to 21-day period at doses that induce functional desensitization). Desensitization of 5-HT_{1A}-mediated inhibition of adenylyl cyclase has also been documented after the administration of clorgyline, tranylcypromine, fluoxetine, and buspirone (see Newman et al., 1993), and the use of molecular biology techniques has allowed us to determine that agonist-induced desensitization of adenylyl cyclase inhibition correlates well with 5-HT_{1A} receptor down-regulation in Swiss 3T3 cells (Van Huizen et al., 1993). However, patterns of desensitization may differ depending on the host cell used to express the receptor, as well as on the response being investigated. Indeed, desensitization of the inhibitory effect of 5-HT_{1A} receptor activation on cAMP production is linked to receptor phosphorylation by PKC in Chinese hamster ovary cells (Raymond, 1991) and by G protein-coupled receptor kinases in insect Sf9 cells (Nebigil et al., 1995). It is unlikely then that results obtained in non-neuronal cell lines may be directly extrapolated to 5-HT neurons. Furthermore, since 5-HT_{1A} receptors that control the firing activity of these neurons are linked (via a G protein) to K^+ and Ca^{2+} channels (Innis and Aghajanian, 1987; Penington et al., 1991, 1993, Penington and Fox, 1994), it is not certain that they possess the same desensitization mechanisms as receptors linked to adenylate cyclase. Alternatively, it has been proposed that antidepressant-induced desensitization of 5-HT_{1A}-mediated responses could be mediated at the signal-transducing (G protein) level (Lesch et al., 1991, 1992; Lesch and Manji, 1992; Chen and Rasenick, 1995). Sustained fluoxetine and clorgyline administration have been found to respectively decrease $G_{\alpha s}$ and increase $G_{\alpha 12}$ mRNA in rat midbrain (Lesch et al., 1992; Lesch and Manji, 1992). Finally, another possible target for antidepressant-induced desensitization of somatodendritic 5-HT_{1A} receptor-mediated control of 5-HT neuron firing are the effector channels to which the receptor is linked by the G protein. The effect of sustained 5-HT_{1A} receptor activation on K^+ channels has been studied on p-neurons of the leech CNS, where they induce phosphorylation of two different types of K^+ channels, increasing their open-state probability (Goldermann et al., 1994). Similar to the above-mentioned restrictions, it may not be concluded that such a mechanism might account for desensitization of 5-HT_{1A}-mediated responses in mammalian 5-HT neurons. However, this as well as the previous observations open new research avenues that would be worth exploring.

C. 5-HT Release

1. Neurotransmitter Release and Its Regulation: Cellular and Molecular Aspects. Exocytosis is the main mechanism used by neuronal cells for releasing neurotransmitter molecules. By this process, synaptic vesicles fuse with the plasma membrane and the neurotransmitter(s) contained within them reach(es) the synaptic cleft. Exocytosis is triggered by cell depolarization. Depolarization induces opening of voltage-sensitive calcium channels and subsequent Ca^{2+} entry. Achieving localized concentrations of 10 to 100 μM around the open channel (Smith and Augustine, 1988), the intracellular Ca^{2+} increase constitutes the major coupling signal that links depolarization and exocytotic secretion (reviewed by Burgoyne and Cheek, 1995). One of the main characteristics of neurotransmitter release is its high speed, the complete cycle being achieved in hundreds of milliseconds. This is apparently due to the fact that secretory vesicles are already docked to the plasma membrane so that when Ca^{2+} entry takes place, vesicles in a close vicinity of activated calcium channels will immediately void their content into the synaptic cleft by formation of a fusion pore (see Burgoyne and Cheek, 1995). Docking and fusing of the vesicles to the plasmalemma is achieved by Ca^{2+}-sensitive vesicle membrane proteins (Augustine et al., 1985; Smith and Augustine, 1988; Leveque et al., 1992). One of these proteins, synapsin I, in an unphosphorylated state, fixes secretory granules to the cytoskeleton. Once it undergoes Ca^{2+}-calmodulin/cAMP-dependent phosphorylation, it releases the vesicles from the cytoskeletal network and allows them to move to the presynaptic membrane (Valtorta et al., 1992) where they will be ultimately docked and voided to the extracellular space. The cytoskeleton is not likely to be involved in a first burst of release, which usually empties already docked vesicles, yet releasing the bound granules from the actin network will facilitate their subsequent recruitment by the plasma membrane in

preparation for the arrival of the next axon potential (see Burgoyne and Cheek, 1995).

The amount of neurotransmitter released is subject to receptor-dependent regulation that may theoretically occur at any stage in the release process described above. However, because most of the exocytotic steps are regulated by Ca^{2+} influx in an exponential manner (Augustine and Charlton, 1986), relatively small changes in its influx will be expected to produce profound changes in neurotransmitter release. Serotonin has been shown to enhance release by increasing voltage-activated Ca^{2+} currents in postsynaptic neurons in the substantia nigra (*pars* compacta) and spinal cord (Nedegaard et al., 1988; Berger and Takahashi, 1990). In contrast, the main autoregulatory effect of 5-HT on its own release is inhibitory. An inhibition in Ca^{2+} influx may be produced either by a direct effect on Ca^{2+} channels (shift in the voltage of activation of the channel; Bean, 1989) or by reducing the depolarization time during which Ca^{2+} enters the cell. This reduction in depolarization duration is frequently achieved by speeding the activation of K^+ or Cl^- currents that end depolarization linked to the action potential (Berlardetti and Siegelbaum, 1988; Brezina and Erxleben, 1988). Serotonin, acting on 5-HT_{1A} autoreceptors, has been shown not only to reduce a high threshold Ca^{2+} current (Penington and Kelly, 1990; Penington et al., 1991; Penington and Fox, 1994), but also to increase conductance to potassium ions (Aghajanian and Lakoski, 1984; Yoshimura and Higashi, 1985). It is important to realize that the experiments assessing the cellular and molecular mechanisms of the regulation of neurotransmitter release have been performed on the cell body, and that there is no direct electrophysiological information on the mechanism by which terminal 5-HT autoreceptors modify Ca^{2+} influx. Linkage via G protein of neurotransmitter receptors to Ca^{2+} or K^+ channels is widely used in nature as a means of inhibiting neurotransmitter release (see Miller, 1990; Anwyl, 1991). It could therefore be a possible mechanism by which 5-HT autoreceptors regulate release from 5-HT terminals. On the other hand, agents like cAMP which promote Ca^{2+}-independent phosphorylation of synapsin I (Bähler and Greengard, 1987) might induce regulation of neurotransmitter release without modifying Ca^{2+} influx to the cell. Indeed, this would be in keeping with not only the observation that adenylate cyclase inhibition by $5\text{-HT}_{1B/1D}$ receptor activation reduces 5-HT release, but also with the fact that cAMP analogs and stimulation of adenylate cyclase by forskolin increase 5-HT release from brain slices and neuroectodermal cell cultures (Schlicker et al., 1987; Tamir et al., 1990).

Neurotransmitter release from neuronal structures has been classically studied in terms of exocytosis, and it is only recently that carrier-mediated release has been considered as a functionally acceptable mechanism for increasing extracellular concentration of a wide number of neurotransmitters including 5-HT (Levi and Raiteri, 1993). Unlike exocytosis, carrier mediated-release is Na^+ but not Ca^{2+} dependent, does not rely on a vesicular but on a cytoplasmic pool of neurotransmitter, is not modulated by presynaptic receptors, and is blocked by uptake inhibitors (see Levi and Raiteri, 1993). Indeed, based on the fact that the 5-HT transporter moves 5-HT with Na^+ and Cl^- across the membrane in one step and K^+ repositions the carrier in a second step (Keyes and Rudnick, 1982; Nelson and Rudnick, 1982; Rudnick, 1986), Rudnick and Wall (1992b,c) have recently shown that purified platelet plasma membrane vesicles, containing the same 5-HT transporter as the one responsible for 5-HT uptake into presynaptic nerve endings (Lesch et al., 1993c), may either accumulate or extrude [^{3}H]5-HT when appropriate transmembrane ionic gradients are imposed. If manipulating the Na^+ or K^+ electrochemical gradient changes the direction of neurotransmitter flow, it would then be tempting to assume that 5-HT may be released via this process during depolarization. Such a mechanism could explain observations by McKenna et al. (1991), in which Ca^{2+}-independent 5-HT release from rat brain synaptosomes was induced by high extracellular KCl concentrations. However, K^+-induced, Ca^{2+}-independent 5-HT release was not confirmed by Berger et al. (1992) using a similar in vitro preparation.

On the other hand, multiple studies have confirmed that the 5-HT transporter mediates 5-HT release induced by substituted amphetamines such as PCA, the anorexigenic drug fenfluramine, MDA and the drug of abuse "ecstasy" (MDMA; McKenna et al., 1991; Berger et al., 1992; Rudnick and Wall, 1992a,c; Sabol et al., 1992; Gu and Azmitia, 1993; Bonanno et al., 1994). Apart from releasing 5-HT, these drugs also cause a degeneration of fine 5-HT terminals (Mamounas and Molliver, 1988; O'Hearn et al., 1988; Molliver and Molliver, 1990). Therefore, transporter-mediated release of 5-HT has been proposed as a possible neurotoxic mechanism for 5-HT neurons, and different properties of 5-HT carriers in fine and beaded axons could account for the differential effect of these drugs on the two types of 5-HT fibers. More recently, the demonstration that nonneurotoxic amphetamine derivatives also induce transporter-mediated 5-HT release strongly suggests that release through the 5-HT transporter is not sufficient to cause destruction of 5-HT terminals (Rudnick and Wall, 1993). Indeed, several studies indicate that the DA-releasing property of these amines may be a necessary cofactor for 5-HT fiber destruction (Stone et al., 1988; Schmidt et al., 1990; Johnson and Nichols, 1991).

2. Physiological Role of Extracellular 5-HT Bioavailability in Midbrain Raphe Nuclei. It is important to bear in mind that all of the methods presently used to determine neurotransmitter release, such as synaptosomal or slice superfusion, in vitro or in vivo voltametry and microdialysis, measure not only the release but also the summation of neurotransmitter release, uptake, diffu-

sion, and metabolism, generally referred to as neurotransmitter output. Hence, whenever the term release is used, it is understood that it does not strictly refer to 5-HT release but rather to what is detected by the method used. With this in mind, the extracellular concentration of 5-HT in the DRN has been estimated to be between 3 and 10 nM using in vivo voltametry and microdialysis experiments (Crespi et al., 1988; Bel and Artigas, 1992; Adell et al., 1993). There seems to be general agreement on the fact that extracellular 5-HT levels may vary according to behavioral state changes (Cespuglio et al., 1990; Houdouin et al., 1991; Portas and McCarley; 1994). However, there is still controversy with regard to the direction of these changes. On the one hand, Jouvet's group has observed the highest 5-hydroxindole peak during sleep (Cespuglio et al., 1990; Houdouin et al., 1991), thus supporting the idea that enhanced 5-HT_{1A} autoreceptor activation secondary to dendritic 5-HT release triggered by hypnogenic factors such as CLIP or VIP (El Kafi et al., 1994) might be responsible for determining the decrease in 5-HT neuron firing activity observed during SWS and REM (see Jacobs and Azmitia, 1992; Jacobs and Fornal, 1993). On the other hand, Portas and McCarley (1994) argue that extracellular 5-HT in the DRN is highest during wakefulness, lowest during REM, and that extracellular somatodendritic 5-HT availability depends directly on the serotonergic action potential activity. The latter observations are in agreement with results from electrophysiological studies in which 5-HT_{1A} antagonists were more effective in increasing 5-HT neuron firing activity during wakefulness than during sleep (Fornal et al., 1994a,b). Furthermore, in vitro neurochemical studies indicate that within a range of 5 to 100 Hz, higher stimulation frequencies elicit increasingly higher extracellular concentrations of 5-HT, as measured by fast cyclic voltametry in rat DRN slices (O'Connor and Kruk, 1991).

The role of firing activity on 5-HT release in the raphe nuclei has also been studied using the Na^+ channel blocker tetrodotoxin (TTX). The local administration of TTX into the DRN or MRN was shown to decrease spontaneous [^{3}H]5-HT release in nonanaesthetized encéphale-isolé cats (Héry et al., 1986) and freely moving rats (Bosker et al., 1994), suggesting that 5-HT release is mainly dependent on firing activity. Other studies did not confirm this view. TTX injection in the immediate vicinity of the DRN (lateral boundaries) did not change the extracellular concentration of 5-HT (Adell et al., 1993). The 5-hydroxyindole acute acid (5-HIAA) peak in the DRN was not altered by intraraphe administration of this Na^+ channel blocker at a concentration that effectively reduced the voltametric signal in the striatum (Scatton et al., 1985). Although it could be argued that TTX injected in the vicinity and not within the raphe nuclei might have not reached 5-HT neurons, and that extracellular 5-HIAA may not always reflect 5-HT release, results from in vitro experiments have also been variable. When TTX was introduced into the perfusion medium, spontaneous [^{3}H]5-HT release from midbrain raphe slices was increased (Héry et al., 1986), electrically evoked release of 5-HT was reduced (Starkey and Skingle, 1994), and K^+-induced release of the neurotransmitter was unaffected (El Mansari and Blier, 1996; Piñeyro and Blier, 1996). Interestingly, Pan et al. (1989) have shown that the electrical stimulation of midbrain raphe slices elicits a multicomponent postsynaptic potential in which a fast EPSP precedes the slow inhibitory postsynaptic potential produced by 5-HT_{1A} receptor activation. This observation was interpreted by the authors as an indication that at least some of the 5-HT is released as a result of synaptically induced excitation of cell bodies, an assumption that seems confirmed by the fact that in this sort of paradigm TTX abolishes not only electrically evoked 5-HT release (Starkey and Skingle, 1994) but also the multiple postsynaptic potential (Pinnock, 1992). On the other hand, TTX resistance of K^+-induced 5-HT release may be explained by 1) K^+-induced depolarization, which triggers a direct activation of voltage-sensitive Ca^{2+} channels and exocytotic release of 5-HT (experiments to determine the effect of Ca^{2+} channel blockers on K^+-induced 5-HT release in the presence and absence of TTX may help to assess this possibility) and/or 2) an increase in extracellular K^+, which may induce carrier-mediated 5-HT release. In favor of the latter mechanism is the observation that 5-HT receptor agonists are much less potent in inhibiting K^+ than electrically induced 5-HT release from raphe nuclei (Middlemiss, 1987; Starkey and Skingle, 1994; Piñeyro et al., 1995b; Piñeyro and Blier, 1996). Furthermore, in view of the fact that pretreatment with reserpine does not modify 5-HT release in the DRN of freely moving rats, release from a cytoplasmic pool has been proposed (Adell et al., 1993). However, it is worth noting that Ca^{2+} omission greatly reduces or even abolishes electrically and K^+-induced 5-HT release (Kerwin and Pycock, 1979; Héry et al., 1986; Starkey and Skingle, 1994; Piñeyro et al., 1995b) from midbrain raphe nuclei, thus indicating that exocytosis is the main mechanism involved in neurotransmitter release in this area. Also, in the cat nodose ganglia, which contain cell bodies and dendrites but no 5-HT axons, K^+-induced 5-HT release is totally abolished in a Ca^{+2}-free medium (Fueri et al., 1984). Such an observation is in keeping with the idea not only that soma and dendrites of 5-HT neurons may release 5-HT, but also that somatodendritic release of 5-HT is predominantly exocytotic.

Considerable evidence supports the view that 5-HT release in the cell body area may be regulated by firing-controlling 5-HT_{1A} autoreceptors. In in vitro superfusion experiments, the 5-HT_{1A} agonists 8-OH-DPAT, buspirone and ipsapirone have been shown to inhibit electrically evoked [^{3}H]5-HT release from rat midbrain raphe slices, and this effect was blocked by 5-HT_{1A} antagonists

such as NAN-190, WAY 100135, and S-UH-301 (Starkey and Skingle, 1994; Davidson and Stamford, 1995a; Piñeyro et al., 1995b; Piñeyro and Blier, 1996). It has also been shown that WAY 100135 increases electrically evoked release of [^{3}H]5-HT from midbrain slices in both the absence and presence of a selective 5-HT reuptake blocker (Starkey and Skingle, 1994; Davidson and Stamford, 1995b), an effect that has been attributed to the blockade of tonic activation of 5-HT$_{1A}$ autoreceptors by the endogenous neurotransmitter. Moreover, local decrease in extracellular 5-HT has also been observed following 8-OH-DPAT injection into the median raphe region (Bosker et al., 1994), further supporting the notion that somatodendritic 5-HT release is subject to local feedback mechanisms through 5-HT$_{1A}$ autoreceptors. Activation of somatodendritic 5-HT$_{1A}$ autoreceptors has a major impact on the amount of 5-HT released not only in raphe nuclei but also in forebrain projection areas. For example, infusion of the 5-HT$_{1A}$ agonist 8-OH-DPAT into the DRN results in a decrease of 5-HT release in the striatum (Bonvento et al., 1992) and the hippocampus (Hutson et al., 1989, Sharp et al., 1989, Adell et al., 1993). Similarly, if the extracellular availability of 5-HT in the biophase of somatodendritic 5-HT$_{1A}$ receptors is increased by the direct application of the neurotransmitter or intraraphe perfusion of SSRIs or MAOIs, 5-HT release in cortex, striatum, and hippocampus is markedly reduced (Becquet et al., 1990; Adell and Artigas, 1991; Celada and Artigas, 1993). If the injection volume within a specific site is small enough to ensure no diffusion to the corresponding neighboring dorsal or MRN, then reduction of 5-HT release will follow the differential projection pattern of the nucleus into which the agonist was administered: injection of 8-OH-DPAT into the MRN reduces the extracellular 5-HT concentration in the hippocampus but not that of the striatum, and the converse is true for intraDRN administration of 8-OH-DPAT (Bonvento et al., 1992; Kreiss and Lucki, 1994).

As is the case with firing activity, the effect of blocking the somatodendritic 5-HT$_{1A}$ autoreceptor may be variable on 5-HT release in projection areas and may in part depend on the extracellular concentration of 5-HT at the cell body level. In unanesthetized cats and freely moving rats, systemic administration of drugs that block 5-HT$_{1A}$ receptors, such as methiothepin, S-UH-301, (+)-WAY 100135, and pindolol, produce no change in striatal or hippocampal 5-HT release (Becquet et al., 1990; Nomikos et al., 1992; Routledge et al., 1993; Romero et al., 1996). In contrast, the systemic administration of S-UH-301 or pindolol before the local infusion of citalopram into the DRN or its systemic administration blocks the reduction caused by the latter drug in hippocampal and striatal 5-HT release (Hjorth, 1993; Romero et al., 1996). Furthermore, infusion of methiothepin into the DRN blocks the reduction in hippocampal 5-HT release caused by systemic administration of the SSRI sertraline (Invernizzi et al., 1991), and systemic administration of the 5-HT$_{1A/1B}$ antagonist (−)-penbutolol (Hjorth and Sharp, 1993) prevents the decrease in hippocampal 5-HT output caused by systemic citalopram administration (Hjorth, 1993). 5-HT$_{1A}$ ligands with low intrinsic activity, such as BMY 7378 and NAN-190, have been shown to produce a small decrease in hippocampal 5-HT. This effect has been attributed to 5-HT$_{1A}$ receptor activation (Hjorth and Sharp, 1990; Sharp et al., 1990). Systemic administration of the partial agonist SDZ 216525 also reduces 5-HT release in terminal projection areas (Sharp et al., 1993b, Gurling et al., 1993). On the other hand, (+)-WAY 100135, which has been shown to inhibit 5-HT neuron firing activity by blocking α_1 adrenoceptors, does not modify hippocampal 5-HT release when given systemically (Routledge et al., 1993).

The idea that, apart from somatodendritic autoreceptors, 5-HT$_{1A}$ receptors not located on the somata of 5-HT neurons may regulate firing-dependent terminal 5-HT release has now gained considerable support. In fact, inactivation of autoreceptors by intraraphe infusion of pertussis toxin prevents the reducing effect on striatal 5-HT release caused by direct administration of citalopram into the dorsal raphe but does not interfere with the reducing effect caused by the systemic administration of 8-OH-DPAT (Romero et al., 1994a). In another line of evidence, electrophysiological experiments in which 5-HT$_{1A}$ agonists were microiontophoretically applied onto 5-HT neurons in the DRN and MRN indicate that the former are more responsive than the latter to the local application of such drugs (Blier et al., 1990). However, following systemic administration of 8-OH-DPAT (250 μg/kg), no differential effect on 5-HT released in dorsal and median raphe-innervated areas was observed (Hjorth and Sharp, 1991). Both observations could be reconciled by admitting that post- and not presynaptic 5-HT$_{1A}$ receptors were being stimulated. On the other hand, also using systemic administration, Sinton and Fallon (1988) have reported a differential sensitivity of dorsal and median raphe neurons to this 5-HT$_{1A}$ agonist: only 5 μg/kg of 8-OH-DPAT was needed to abolish 5-HT neuron firing activity in the DRN, whereas 30 μg/kg was not enough to induce cessation of firing of 5-HT neurons in the MRN. Given the differences in doses used, it could be possible that pre- and postsynaptic receptors were differently stimulated in the two studies.

3. Autoregulation of 5-HT Release in the Raphe Nuclei. The above-mentioned evidence indicates that, by means of a potent feedback control mechanism on 5-HT neuron firing frequency, somatodendritic 5-HT$_{1A}$ autoreceptors constitute a major presynaptic determinant in the efficacy of 5-HT synaptic transmission. Hence, all auto- or heteroregulatory influences on 5-HT availability in the biophase of 5-HT$_{1A}$ autoreceptors will contribute to determine the overall efficacy of 5-HT synaptic transmission in the brain.

Apart from being regulated by somatodendritic 5-HT_{1A} autoreceptors, 5-HT release from 5-HT neurons is controlled by terminal 5-$HT_{1B/1D}$ autoreceptors (see Starke et al., 1989; Table 4). These receptors, unlike the somatodendritic ones, can modify 5-HT release without altering 5-HT neuron firing activity (e.g., see Crespi et al., 1990). There is now considerable evidence indicating that this firing-independent control of 5-HT release also takes place at the cell body level and that non-5-HT_{1A} receptors are involved. Using in vivo voltametry, Blier et al. (1990) have shown that the systemic administration of the 5-HT_1 agonist RU 24969 could reduce extracellular availability of 5-hydroxindoles in the DRN of anesthetized rats without altering 5-HT neuron firing frequency. More recently, these results have been confirmed and extended using TFMPP which, following its systemic administration, was also shown to reduce extracellular availability of hydroxindoles in the rat DRN without modifying 5-HT neuron firing activity (Piñeyro et al., 1995, 1996b). Furthermore, the reducing effects of TFMPP and RU 24969 on extracellular availability of 5-hydroxyindoles in the rat DRN are blocked by the non-5-HT_{1A} agonist mianserin but not by WAY 100135. Evidence indicating that 5-HT release in the DRN may be controlled independently of 5-HT_{1A} receptor activation has also been gathered using an electrophysiological paradigm. The latter allowed to infer the amount of extracellular 5-HT available to stimulate somatodendritic 5-HT_{1A} autoreceptors by measuring changes in 5-HT neuron firing activity, a direct consequence of 5-HT_{1A} receptor activation, following electrical stimulation of the medial forebrain bundle in the VTA. The 5-HT agonists TFMPP and RU 24969 were shown to reduce the duration of suppression of 5-HT neuron firing evoked by 5-HT pathway stimulation without modifying their basal electrical activity. The effect of these agonists was blocked by (−)-propranolol and mianserin (which do not interfere with 5-HT_{1A}-mediated electrophysiological responses of 5-HT neurons). This observation was interpreted as further indication that non-5-HT_{1A} agonists regulate 5-HT release in the DRN.

Results from in vitro superfusion studies using midbrain raphe slices from guinea pigs also support this idea: 1) the 5-$HT_{1B/1D}$ antagonist GR 127935 increases electrically evoked release of 5-HT in guinea pig raphe slices (Starkey and Skingle, 1994; El Mansari and Blier, 1996), 2) the 5-$HT_{1D/1B}$ agonist sumatriptan inhibits 5-HT release and this effect, not altered by (+)-WAY 100135, is blocked by the 5-$HT_{1B/1D}$ antagonist GR 127935 which also blocks the inhibitory effect of the nonselective agonist 5-methoxytryptamine (5-MeOT). However, neither sumatriptan- nor 5-MeOT-mediated

TABLE 4

Pharmacological profile of 5-HT terminal autoreceptors in cortex and hippocampus of different species as determined by their effect on 5-HT release

Species	Agonists	Antagonists	Reference
Cortex			
Rat	5-CT = RU 24969 > 5-HT >>> 8-OH-DPAT	methio. > meterg.	Limberger et al., 1991
	5-HT	(±)-cyanop. < (−)-alprenolol < (−)-pind. < (−)-oxprenolol	Middlemiss, 1986
		(−)-prop.	Middlemiss, 1984
		(−)-penbutolol	Hjorth and Sharp, 1993
	5-CT > 5-HT > 5-MeOT > LSD	(−)-cyanop. > (±)-cyanop. > methio. > (+)-cyanop. > (±)-prop. > (−)-pind. > meterg. > quipazinel	Engel et al., 1986
		(±)-cyanop. < methio. < (−)-prop. < (−)-pind.	Schlicker et al., 1985
Pig	5-HT > 5-MeOT > RU 24969 ≫ 8-OH-DPAT	methio. > meterg. ≫ mianserin > prop. > mesulergine	Schlicker et al., 1989
Guinea pig	5-CT > 5-HT > RU 24969 sumatriptan	methio. > meterg. >>> prop.	Limberger et al., 1991 Sleight et al., 1990
	5-CT > 5-HT	meterg.	Ormandy, 1993
	5-MeOT > sumatriptan		
	L694247		Beer et al., 1993
	5-CT > 5-HT > sumatriptan	methio.	Wilkinson and Middlemiss, 1992
	5-CT > 5-MeOT		Blier and Bouchard, 1994
	5-MeOT > sumatriptan	methio. > meterg. ≫ methysergide	Middlemiss et al., 1988
		GR 127935	El Mansari and Blier, 1996
		GR 127935 > methio.	Price et al., 1994
Rabbit	5-CT > 5-HT > RU 24969	methio. > meterg. >>> prop.	Limberger et al., 1991
Human	RU 24969 > 5-CT	methio. > methysergide > meterg. = prop.	Galzin et al., 1992
	5-HT > sumatriptan	methio. > meterg.	Maura et al., 1993
Hippocampus			
Rat	RU 24969 < 5-HT	methio. > (−)-prop.	Maura et al., 1986
	RU 24969 > CP 93129	methio.	Hjorth and Tao, 1991
Guinea pig	5-HT > sumatriptan	methio.	Wilkinson et al., 1993
	5-CT > 5-MeOT		Blier and Bouchard, 1994
Rabbit	5-CT > 5-HT > 5-MeOT >>> 8-OH-DPAT	meterg. > cyanop. > methio.	Feuerstein et al., 1987

Within each species drugs have been placed in a visual analog scale (decreasing affinity to the right). cyanop., cyanop indolol; methio., methiothepin; prop., propranolol; pind., pindolol; meterg., metergoline.

responses are blocked by WAY 100135 (Starkey and Skingle, 1994; El Mansari and Blier, 1996; note: 5-$HT_{1B/1D}$ nomenclature used in this manuscript conforms to new guidelines adopted by the International Nomenclature Committee[3]).

According to the new nomenclature, receptors predominantly found in guinea pig brain are of the 5-HT_{1B} type, with a nonrodent pharmacological profile (Heuring et al., 1986; Bruinvels et al., 1993). Since drugs used to characterize regulation of somatodendritic 5-HT release by non-5-HT_{1A} receptors in guinea pig brain do not readily discriminate 5-HT_{1B} from 5-HT_{1D} receptors in this species, and the former largely outnumber the latter, it seems reasonable to postulate that newly named 5-HT_{1B} receptors negatively regulate 5-HT release in guinea pig midbrain raphe nuclei. Interestingly, CP93129, a drug that has been characterized as a 5-HT_{1B} agonist in rodents, produced no effect in electrically evoked release from midbrain guinea pig slices (El Mansari and Blier, 1996). It is not possible for the time being to determine whether the lack of effect of this drug is due to the fact that guinea pig 5-HT_{1B} receptors do not recognize CP93129 as rodent 5-HT_{1B} receptors do, or to the fact that 5-HT_{1D} and not 5-HT_{1B} receptors control somatodendritic 5-HT release in the guinea pig brain.

In rodents, where brain 5-HT_{1D} as well as 5-HT_{1B} receptors have been detected (Hoyer et al., 1985a; Waeber et al., 1989a; Bruinvels et al., 1993), superfusion studies performed in midbrain raphe slices of rats and mice (Piñeyro et al., 1995a, 1996b) indicated that 5-HT_{1B}/5-HT_{1D}-like receptors negatively regulate 5-HT release in this brain area. Evidence accumulated in the above-mentioned studies may be separated into two categories: 1) Observations that support a role for 5-HT_{1D}-like receptors in the regulation of 5-HT release in anterior raphe nuclei: the 5-HT_1 agonist 5-carboxyamidotryptamine (5-CT) induced a concentration-dependent inhibition of the electrically evoked release of [^{3}H]5-HT from preloaded rat raphe slices which was partly blocked by the 5-$HT_{1B/1D}$ antagonists GR 127935 and mianserin which in rats has a very high selectivity for 5-HT_{1D} versus 5-HT_{1B} receptors; the 5-$HT_{1B/1D}$ agonist sumatriptan inhibited electrically evoked release of [^{3}H]5-HT from rat and wild-type mice raphe slices, and this effect was blocked by mianserin and GR 127935; and midbrain slices from 5-HT_{1B} knockout mice maintained a pharmacological response similar to that observed in wild types. 2) Observations that rule out other specific receptor subtypes include: the fact that the effect of sumatriptan was not blocked by S-UH-301 nor (+)-WAY 100135 excludes a possible nonselective activation of 5-HT_{1A} receptors, and in spite of its inhibitory effect on evoked [^{3}H]5-HT release in terminal regions, the lack of effectiveness of the selective 5-HT_{1B} agonist CP 93129 to inhibit evoked release from rat and mice midbrain slices strongly suggests that 5-HT_{1B} receptors are not involved in the sumatriptan-mediated response in midbrain raphe. Rat (as well as human) 5-HT_{1E} and 5-HT_{1F} receptors have low affinity for 5-CT, which in our studies effectively mimicked sumatriptan mediated responses. Similarly, 5-HT_{1E} sites do not bind sumatriptan with high affinity. Studies from human brain indicate that 5-HT_{1F} sites have low affinity for methiothepin and do not bind propranolol, both of which blocked the effect of sumatriptan on 5-HT release in rodent midbrain slices. Hence, in spite of the fact that sumatriptan binds to 5-HT_{1F} receptors, it is unlikely that these or the 5-HT_{1E} subtype may have mediated the sumatriptan-induced inhibition of 5-HT release from rodent brain. Sumatriptan and GR 127935 have very low affinity for murine 5-$HT_{5A/5B}$ sites. The affinity of sumatriptan for rat 5-HT_7 receptors is also considerably low (although higher than for 5-HT_5). If 5-HT_7 receptors played an important role in inhibiting 5-HT release in midbrain nuclei, the inhibitory effect of 8-OH-DPAT (that binds with higher affinity to 5-HT_7 than to 5-HT_{1D} receptors) on somatodendritic 5-HT release would have been greater, not smaller, than that of sumatriptan. Hence, even if mRNAs for 5-HT_{1D}, 5-HT_5, and 5-HT_7 receptor subtypes are present in the DRN, the 5-HT_{1D} receptor, or a subtype bearing a similar pharmacology, was deemed a better choice to account for the effect of different drugs tested on 5-HT release from rat midbrain raphe (see Table 5 for pharmacology of G protein-coupled 5-HT receptors).

In contrast with the above-mentioned observations on the role of 5-HT_1 receptors controlling 5-HT release in the DRN, Davidson and Stamford (1995a) have reported that CP 93129 inhibited in vitro 5-HT release from rat DRN. On the other hand, they have also observed that the effect of CP 93129 was only blocked at scattered time points during continuous superfusion with the 5-HT_{1B} antagonist isamoltane, an unexpected response since once the blockage is established, if there are no changes in drug concentrations there is no reason for such fluctuation. Furthermore, the stimulation protocol used in

[3] The International Nomenclature Committee has named two closely related recently cloned 5-HT receptors 5-HT_{1D} and 5-HT_{1B}. Initially, and for a brief period of time, these receptors were referred to as 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. The 5-$HT_{1D\beta}$ is the receptor that the Nomenclature Committee now calls 5-$HT_{1B,}$ and 5-$HT_{1D\alpha}$ is the one that is now named 5-HT_{1D}. The new nomenclature should not be confused with the original pharmacological definition of 5-$HT_{1B/1D}$ receptor subtypes. The pharmacologically defined 5-HT_{1B} receptor was only found in rodents, whereas the 5-HT_{1D} receptor was generally found only in nonrodent species. With the cloning of both receptors, it became clear that 5-HT_{1B} (5-$HT_{1D\beta}$) was not absent from nonrodent species, but that a difference of a single amino acid in transmembrane VII of the rodent receptor caused the large difference in pharmacological rofile. In spite of their pharmacological differences in rodents and nonrodents, these receptors are all classified as 5-HT_{1B}, irrespective of the species they appear in, since they are homolog receptors across species. Because the currently-defined 5-HT_{1D} receptor (5-$HT_{1D\alpha}$) appears to be a receptor of low expression density, it seems likely that the receptor originally defined in pharmacological terms as 5-HT_{1D} is the receptor briefly known as 5-$HT_{1D\beta}$ and is now called 5-HT_{1B}.

TABLE 5
Affinity values of G protein-coupled 5-HT receptors for different 5-HT drugs[a]

	5-HT$_{1A}$	5-HT$_{1B}$[b]	5-HT$_{1D}$[b]	5-HT$_{1E}$	5-HT$_{1F}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_{4}$	5-HT$_{5A/B}$	5-HT$_{6}$	5-HT$_{7}$
			0.7 nM[19]								
		7 nM[17]	0.37 nM[13]					1,000 nM[8]	16–40 nM[4]	774 nM[6]	0.12 nM[5]
5-CT	0.2 nM[31]	0.35 nM[13]	1.5 nM[29]	7,875 nM[3]	717 nM[3]	2,000 nM[31]	600 nM[31]	3,160 nM[44]	12–235 nM[9]	253 nM[8]	0.93 nM[8]
		5 nM[14]									
	79 nM[35]	11 nM[13]	0.6 nM[13]					>10,000 nM[8]	16–100 nM[4]		
Methiothepin	89 nM[36]	50 nM[35]	50 nM[35]	194 nM[3]	652 nM[3]	1.58 nM[35]	25 nM[35]		23–145 nM[9]	0.39 nM[6]	3.7 nM[8]
			120 nM[19]								
8-OH-DPAT	1.2 nM[24]	>10,000 nM[13,14]	99 nM[13]				>10,000 nM[43]	>10,000 nM[44]	400–1,200 nM[4]		
	0.4 nM[36]		975 nM[15]	3,333 nM[3]	1,772 nM[3]	700 nM[24]					52 nM[5]
		>10,000 nM[31]					>10,000 nM[31]				
WAY 100135	23 nM[32]					6,000 nM[31]					
	52 nM[32]	>10,000 nM[35]									
S-UH-301	126 nM[34]					7,200 nM[35]	7,150 nM[35]				
		8 nM[12]	1,100 nM[18]								
CP 93129	1,500 nM[12]	55 nM[16]	954 nM[40]			7,200 nM[12]	2,900 nM[12]				
		55 nM[35]	1,509 nM[15]								
		5 nM[13]	1,300 nM[13]	>10,000 nM[18]	>10,000 nM[18]	2,987 nM[33]			6,000–20,000 nM[4]		
(−)-Propranolol	52.5 nM[35]	50 nM[40]	57 nM[16]			158 nM[42]	200 nM[33]				
		465 nM[16]									
	320 nM[29]	45 nM[13]	9.5 nM[13]			>10,000 nM[22]	>10,000 nM[22]		8,000–15,000 nM[4]		506 nM[5]
Sumatriptan	239 nM[38]	100 nM[14]	8.9 nM[15]	2,520 nM[3]	23 nM[3]						951 nM[8]
GR 127935	125 nM[31]	3 nM[30]	3 nM[30]	3,000–4,000 nM[30]		40 nM[30]	630 nM[30]	>10,000 nM[30]	6,000–20,000 nM[30]		
	500 nM[13]										
	1,000 nM[35]	6,000–10,000 nM[13,35]	30 nM[13]				10 nM[35]				
Mianserin	1,150 nM[39]		398 nM[35]			7.9 nM[35]	1.9 nM[41]			38 nM[6]	67 nM[5]
	200 nM[35]										
	300 nM[23]	3.3 nM[13]	59 nM[13]			32 nM[24]	13 nM[35]		3,000–4,000 nM[4]		
TFMPP	170 nM[24]	27 nM[24]	690 nM[35]	6,293 nM[3]	1,002 nM[3]	780 nM[35]	50 nM[23]			482 nM[6]	466 nM[8]
		1.22 nM[14]									
	7.9 nM[35]	0.36 nM[13]	65.6 nM[15]				316 nM[35]	>10,000 nM[44]	3,000–4,000 nM[4]		
RU 24969	2.5 nM[37]	2 nM[15]	47 nM[13]			42 nM[35]	47.8 nM[38]				
								Renzapride			
	8-OH-DPAT	CP93129	L694247[27]			Ketanserin	Mesulergine	BUIM8			
Selective ligand	WAY 100635[28]	SDZ21009[28]	GR172935[28]			MDL 100907[28]	SB200646[28]	GR113808[28]			
	Yes	Yes	Yes	No[2]	Yes				Yes		Yes[5]
mRNA in DRN	Very high[7]	High[2,20]	Moderate[2]		Low[2]				5-HT$_{5B}$[4]		
		No[21]	Yes/low[26]								No[1]
Binding sites in DRN	Yes	Yes/moderate[26]									Yes/moderate[11]
	Very high[7]										

Superscript numbers represent sources: 1, Mengod et al., 1996; 2, Bruinvels et al., 1992; 3, Adham et al., 1993a; 4, Matthes et al., 1993; 5, Ruat et al., 1993; 6, Monsma et al., 1993; 7, Lucas and Hen, 1995; 8, Bard et al., 1993; 9, Erlander et al., 1993; 10, Adham et al., 1993b; 11, To et al., 1995; 12, Kow et al., 1992; 13, Hamblin et al., 1992a; 14, Cerutis et al., 1994; 15, Maenhault et al., 1991; 16, Parker et al., 1993; 17, Oksenberg et al., 1992; 18, Adham et al., 1994; 19, Weinshank et al., 1992; 20, Doucet et al., 1995; 21, Vergé et al., 1986; 22, Glennon et al., 1994; 23, Waeber et al., 1988a; 24, Herrick-Davis and Titeler, 1988; 25, Zgombick et al., 1993; 26, Bruinvels et al., 1993; 27, Heald et al., 1994; 28, Langlois et al., 1993; 29, Koe et al., 1994; 30, Starkey and Skingle, 1994; 31, Skingle et al., 1994; 32, Fletcher et al., 1993; 33, Prisco et al., 1993; 34, Cornfield et al., 1991; 35, Griebel et al., 1995; 36, Gozlan et al., 1983; 37, Peroutka, 1986; 38, Van Wijngaarden, 1990; 39, Hoyer, 1985; 40, Bruinvels et al., 1991; 41, Yagaloff and Hartig, 1985; 42, Alexander and Wood, 1987; 43, Sanders-Bush, 1988; 44, Dumuis et al., 1988.

[a] Affinity values are given as K_D or K_i values for binding studies performed in brain membranes or heterologous expression systems.

[b] Old nomenclature as it appears in original reference.

the latter study and the one from our group (Piñeyro et al., 1995b) were different; in one, stimulation parameters were set to mimic 5-HT neuron firing frequency during wakefulness (1–5 Hz; Piñeyro et al., 1995b), whereas in the other, pseudosingle pulse stimulations (100 Hz) were used (Davidson and Stamford, 1995a). It has been extensively documented that different stimulation parameters may differ in the concentration of 5-HT induced in the biophase of 5-HT receptors, eliciting different drug actions even if all other experimental variables are kept constant (see below). In these studies, however, not all variables were kept constant, the concentration of CP 93129 used by Davidson and Stamford (1995a) was three times as high as the one used in our study (Piñeyro et al., 1995b). In our paradigm, concentrations of this product higher than 100 nM have been shown to produce significant increases in basal 5-HT release. Because values for this parameter were not reported when a concentration of 300 nM CP 93129 was used, it may not be ruled out that evoked release of 5-HT may have been reduced after treatment with the 5-HT_{1B} agonist due to unloading of the slices previous to stimulation.

Based on the observations mentioned above, it could be hypothesized that if in rats 5-HT_{1B} receptors indeed play a regulatory role in midbrain 5-HT release, it would probably involve 5-HT release from collaterals and 5-HT terminals, whereas on the other hand, 5-HT_{1D}-like receptors would control a larger somatodendritic neurotransmitter release. Because 5-HT terminals are not abundant in DRN and MRN, the previous interpretation would lend support to two observations: 1) lack of effect of 5-HT_{1B} receptor blockade following systemic administration of an SSRI to rats (Fig. 2) and 2) lack of effect of 5-HT_{1B} receptor blockade during wakefulness, a time when extracellular somatodendric concentrations of 5-HT are high. The former interpretation would also be in keeping with the observation that even if in binding studies the number of 5-HT_{1B} receptors is much higher than that of 5-HT_{1D} receptors, the activation of the latter by regulating a greater contingent of releasable 5-HT (somatodendritic) produces a dramatic inhibition of release, whereas the activation of the former has a modest or no effect.

Comparing the results obtained in microdialysis studies in rats and guinea pigs has helped evaluate the role of 5-$HT_{1B/1D}$ receptor subtypes in the regulation of 5-HT release in raphe nuclei. In anesthetized rats pretreated with citalopram, the systemic administration of the 5-$HT_{1A/1B}$ antagonist (−)-penbutolol (8 mg/kg s.c) produces an increase in hippocampal 5-HT release (Hjorth, 1993), most likely by simultaneously blocking activated

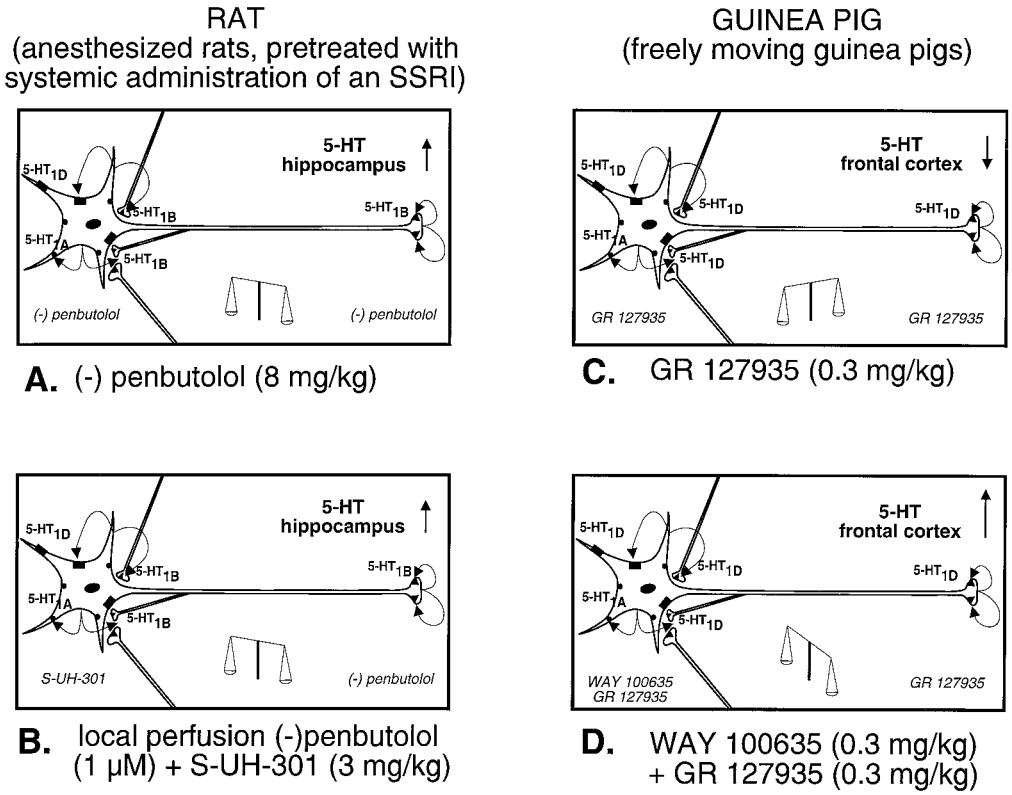

FIG. 2. A, effect of systemic administration of the 5-$HT_{1A/1B}$ antagonist (−)-penbutolol on spontaneous hippocampal 5-HT release in anesthesized rats. B, effect of hippocamal perfusion of the 5-$HT_{1A/1B}$ antagonist (−)-penbutolol and systemic administration of the 5-HT_{1A} antagonist S-UH-301 on spontaneous 5-HT release in rat hippocampus. C, effect of the systemic administration of the 5-$HT_{1B/1D}$ antagonist GR 127935 on cortical 5-HT release of freely moving guinea pigs. D, effect of the combined administration of a 5-HT_{1A} (WAY 100135) and the 5-HT_{1D} antagonist on cortical 5-HT release in freely moving guinea pigs (see text for details).

somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors (Fig. 2A). This effect is similar to that observed following intrahippocampal perfusion of (−)-penbutolol (1 μM in the perfusion medium) in combination with systemic administration of the 5-HT_{1A} antagonist S-UH-301 (3 mg/kg s.c.; Fig. 2B). When administered systemically, there should be no reason why (−)-penbutolol would not block somatodendritic 5-HT_{1B} receptors in addition to terminal ones. Hence, if 5-HT_{1B} receptors played a significant inhibitory role in the regulation of midbrain 5-HT release, the logical expectation would be for this type of treatment to induce an additional increase in terminal 5-HT release as compared to intrahippocampal perfusion of (−)-penbutolol, provided that 5-HT_{1A} autoreceptor function is blocked. Since there was no such observation, the most parsimonious conclusion would be that, in rats, 5-HT_{1B} receptors do not play a major role in controlling extracellular availability of 5-HT at the somatodendritic level. In freely moving guinea pigs, the results observed are quite different (Price et al., 1994). The systemic administration of the 5-$HT_{1B/1D}$ antagonist GR 127935 (0.3 mg/kg) produces a marked decrease in extracellular cortical 5-HT (Fig. 2C), an unexpected effect since this drug had been shown to block terminal 5-HT_{1B} autoreceptors in this species and increase 5-HT release in cortical slices (Price et al., 1994; Table 6). The decrease in release could be due, in theory, to the partial agonistic properties of GR 127935 on guinea pig terminal autoreceptors (Tingley et al., 1994). However, it is improbable that this was the actual cause for reduced in vivo cortical 5-HT release since the systemic administration of WAY 100635 (1 mg/kg) not only blocked, but in fact reversed the inhibitory effect of GR 127935, thus producing a marked elevation in cortical 5-HT levels (Price et al., 1994; Fig. 2D). The reversal by WAY 100635 of the GR 127935 effect can be explained by assuming that GR 127935 blocked 5-$HT_{1B/1D}$ receptors that negatively control 5-HT release at the somatodendritic level. In doing so, such an antagonism would promote a higher extracellular 5-HT level in raphe nuclei and a greater activation of somatodendritic 5-HT_{1A} autoreceptors. This, in turn, would lead to a reduction in 5-HT neuron firing and thus to a reduction in firing-dependent terminal 5-HT release. The administration of WAY 100635 blocks the 5-HT_{1A} autoreceptors, the 5-HT neurons recover their firing activity, and the enhancing effect of terminal 5-HT_{1B} receptor blockade (by GR 127935) on cortical 5-HT release is then unveiled. It would be interesting to test this hypothesis by assessing the effect of systemic administration of GR 127935 in rat brain terminal projection areas. If inhibition resulted, as it did in guinea pigs, this would suggest the blockade of a somatodendritic 5-HT_{1D} receptor. In such a case, it should be determined whether WAY 100635 could unmask the enhancing effect of GR 127935 produced by terminal 5-HT_{1B} receptor blockade. Because rat and guinea pig 5-HT_{1B} and 5-HT_{1D} receptors are species homologs, speculating that the 5-HT_{1D} subtype may be in control of somatodendritic 5-HT release whereas 5-HT_{1B} receptors control terminal release of the neurotransmitter seems plausible. If indeed in humans somatodendritic and terminal autoreceptors are of the 5-HT_{1D} and 5-HT_{1B} subtype, respectively, it might be possible to develop selective terminal and somatodendritic ligands. Theoretically, the expected effect of somatodendritic 5-HT_{1D} activation and of terminal 5-HT_{1B} receptor blockade would be an enhancement of 5-HT release in projection areas.

Concerning the actual localization of 5-HT_{1D} or 5-HT_{1D}-like receptors, the fact that they are functional in midbrain slices indicates that they may be present within the raphe nuclei. Indeed, in slices, the raphe nuclei are separated from their afferent sources. Therefore, the possibility that the receptors in question are located on cell bodies of any of the extra-midbrain afferents to the 5-HT nuclei may be ruled out. Moreover, addition of TTX to the perfusion medium of midbrain raphe slices does not modify the inhibitory effect of sumatriptan on the K^+-evoked release of [^{3}H]5-HT (El Mansari and Blier, 1996; Piñeyro and Blier, 1996), 5-HT_{1D}-like receptors have been proposed to be autoreceptors. Finally, GR 127935 has been shown not to block the inhibitory effect of 5-HT on 5-HT neuron firing activity in guinea pig raphe slices (Craven and Grahame-Smith, 1994), and, in rat DRN, TFMPP as well as RU 24969 reduce somatodendritic availability of 5-hydroxyindoles without changing 5-HT neuron firing activity (Blier et al., 1990; Piñeyro et al., 1996b), further suggesting that in anesthetized animals non-5-HT_{1A}, and possibly 5-HT_{1D} receptors, contribute to the regulation of somatodendritic release of 5-HT independent of the regulation of 5-HT neuron firing. Such an interpretation is further supported by the observation that in anesthetized guinea pigs, a situation in which extracellular somatodendritic availability is comparatively low, systemic administration of GR 127935 leaves 5-HT neuron firing activity unchanged (Sprouse et al., 1995). On the other hand, it would be expected that in freely moving animals systemic GR 127935 administration would reduce 5-HT neuron if not given in combination with a 5-HT_{1A} receptor antagonist.

4. Heteroregulation of Neurotransmitter Release in Midbrain Raphe Nuclei. Numerous neurotransmitters and neuromodulators from afferent terminals or intrinsic non-5-HT neurons influence 5-HT release in the raphe nuclei. For example, low-frequency (1.5 Hz) stimulation of the habenulo-raphe pathway elicits a decrease in 5-HT release in the DRN (Reisine et al., 1982). Similar results are obtained by intraraphe or systemic administration of GABA or GABA agonists (Scatton et al., 1985; Becquet et al., 1990). This pharmacological inhibitory effect on DRN 5-HT release is abolished by transection of the habenulo-raphe pathway (Nishikawa and Scatton, 1985). The need for the integrity of the habe-

TABLE 6

Differential influence of species and stimulation paradigm on the effects of terminal autoreceptor antagonists on 5-HT release in cortex and hippocampus

Species	Antagonist	Effect Observed	Stimulation Procedure Used	Reference
Cortex				
Rat	(−)-Alprenolol (1 μM)	↑	K^+ 25 mM	Middlemiss, 1986
	(−)-Atenolol (1 μM)	↔	K^+ 25 mM	Middlemiss, 1986
	(±)-Cyanopindolol (10–100 nM)	↑	K^+ 25 mM	Middlemiss, 1986
	(±)-Cyanopindolol (10 nM)	↓	3 Hz; 360 pulses	Schlicker et al., 1985
	Isamoltane (1 μM)	↑	100 Hz; 4 pulses	Limberger et al., 1991
	Mesulergine (1 μM)	↔	K^+ 25 mM	Middlemiss et al., 1988
	Metergoline (1 μM)	↔	3 Hz; 360 pulses	Göthert, 1980
	Metergoline (100 nM)	↓	100 Hz; 4 pulses	Limberger et al., 1991
	Methiothepin (1 μM)	↑	K^+ 25 mM	Middlemiss, 1984
	Methiothepin (100 nM)	↑	3 Hz; 360 pulses	Göthert, 1980
	Methiothepin (100 nM)	↔	100 Hz; 4 pulses	Limberger et al., 1991
	Methysergide (1 μM)	↔	K^+ 25 mM	Middlemiss et al., 1988
	(−)-Oxprenolol (1 μM)	↑	K^+ 25 mM	Middlemiss, 1986
	(+)-Oxprenolol (1 μM)	↑	K^+ 25 mM	Middlemiss, 1986
	(−)-Pindolol (1 μM)	↔	K^+ 25 mM	Middlemiss, 1986
	(−)-Propranolol (1 μM)	↔	K^+ 25 mM	Middlemiss, 1984
	(−)-Propranolol (1 μM)	↓	100 Hz; 4 pulses	Limberger et al., 1991
	Quipazinel (10 μM)		K^+ 25 mM	Middlemiss, 1984
Guinea pig	(±)-Cyanopindolol (1 μM)	↔	K^+ 25 mM	Middlemiss et al., 1988
	GR 127935 (10 μ)	↑	Spontaneous release, freely moving	Roberts et al., 1994
	GR 127935 (100 nM)	↑	Spontaneous release, anesthesized	Skingle et al., 1995
	Isamoltane (1 μM)	↑	100 Hz; 4 pulses	Limberger et al., 1991
	Mesulergine (1 μM)	↔	K^+ 25 mM	Middlemiss et al., 1988
	Metergoline (300 nM)	↔		
	Metergoline (1 μM)	↓	100 Hz; 4 pulses	Limberger et al., 1991
	Methiothepin (10 nM)	↔	K^+ 25 mM	Middlemiss et al., 1988
	Methiothepin (300 nM)	↑		Wilkinson et al., 1993
	Methiothepin (1 μM)	↔	100 Hz; 4 pulses	Limberger et al., 1991
	Methysergide (1 μM)	↓	K^+ 25 mM	Middlemiss et al., 1988
	(−)-Propranolol (1 μM)	↔	100 Hz; 4 pulses	Limberger et al., 1991
Rabbit	Isamoltane (1 μM)	↑	100 Hz; 4 pulses	Limberger et al., 1991
	Metergoline (100 nM)	↓	100 Hz; 4 pulses	Limberger et al., 1991
	Methiothepin (1 μM)	↔	100 Hz; 4 pulses	Limberger et al., 1991
	(−)-Propranolol (1 μM)	↔	100 Hz; 4 pulses	Limberger et al., 1991
Hippocampus				
Rat	BMY 7378 (0.25 mg/kg i.v.)	↑	0.5 or 1 Hz; 60 or 120 pulses anesthesized	Chaput and de Montigny, 1988
	(±)-Cyanopindolol (10 nM–μM)	↓	3 Hz; 360 pulses	Feuerstein et al., 1987
	(±)-Cyanopindolol (30–300 nM)	↓	K^+ 15 mM	Maura et al., 1987
	(±)-Cyanopindolol (10–100 nM) + nitroquipazine (1 μM)	↑	3 Hz; 360 pulses	Feuerstein et al., 1987
	(±)-Cyanopindolol (10 nM–1 μM) + fluvoxamine (1 μM)	↑	3 Hz; 360 pulses	Feuerstein et al., 1987
	Mesulergine (300 nM)	↔	K^+ 15 mM	Maura et al., 1986
	Metergoline (1 mg/kg i.v.)	↑	1 Hz; 120 pulses	Haddjeri et al., 1996
	Methiothepin (30–300 nM)	↔	K^+ 15 mM	Maura et al., 1986
	Methiothepin (1 mg/kg i.v.)	↑	5 Hz; 600 pulses anesthesized	Chaput et al., 1986a,b
	Methiothepin (1 mg/kg i.v.)	↑	1 Hz; 120 pulses anesthesized	Chaput et al., 1986a,b
	Methiothepin (3 μM)	↑	K^+ 30 mM	Auerbach et al., 1990
	Methiothepin (10 μM)	↑	Spontaneous release anesthesized	Hjörth and Tao, 1991
	Methysergide (300 nM)	↔	K^+ 15 mM	Maura et al., 1986
	(−)-Penbutolol (1 μM) + citalopram (5 mg/kg s.c.)	↔	Spontaneous release anesthesized	Hjörth, 1993
	(−)-Propranolol (300 nM)	↔	K^+ 15 mM	Maura et al., 1986, 1987
Guinea pig	Methiothepin (300 nM–1 μM)	↑	1 Hz; 120 pulses	Wilkinson and Middlemiss, 1992
Rabbit	(±)-Cyanopindolol (10 nM–1 μM)	↓	3 Hz; 360 pulses	Feuerstein et al., 1987
	(±)-Cyanopindolol (10 nM–1 μM) + nitroquipazine (1 μM)	↓	3 Hz; 360 pulses	Feuerstein et al., 1987
	(±)-Cyanopindolol (10 nM–1 μM) + fluvoxamine (1 μM)	↓	3 Hz; 360 pulses	Feuerstein et al., 1987

TABLE 6
Continued

Species	Antagonist	Effect Observed	Stimulation Procedure Used	Reference
	Metergoline (10 nM–1 μM)	↔	3 Hz; 360 pulses	Feuerstein et al., 1987
	Metergoline (10 nM–1 μM) + nitroquipazine (1 μM)	↑	3 Hz; 360 pulses	Feuerstein et al., 1987
	Metergoline (10 nM–1 μM) + fluvoxamine (1 μM)	↑	3 Hz; 360 pulses	Feuerstein et al., 1987
	Methiothepin (10 nM–1 μM)	↑	3 Hz; 360 pulses	Feuerstein et al., 1987
	Methiothepin (10 nM–1 μM) + nitroquipazine (1 μM)	↑	3 Hz; 360 pulses	Feuerstein et al., 1987
	Methiothepin (10 nM–1 μM) + fluvoxamine (1 μM)	↑	3 Hz; 360 pulses	Feuerstein et al., 1987
	Methysergide (10 nM–1 μM)	↓	3 Hz; 360 pulses	Feuerstein et al., 1987
	(−)-Propranolol (10 nM–1 μM)	↔	3 Hz; 360 pulses	Feuerstein et al., 1987

nulo-raphe connection has thus been interpreted in two alternative ways: 1) the effect of GABA or GABA agonists is an indirect one, and GABA receptors are located on habenulo-raphe fibers or 2) the inhibitory effect of GABA is made evident only if 5-HT neurons have a certain degree of tonic activation, provided in turn by the habenulo-raphe pathway. It is worth noting that the most consistently described tonic excitatory input originates in locus ceruleus (Baraban and Aghajanian, 1981). The common feature shared by both of the interpretations above is that they assume that the habenulo-raphe pathway must contain an excitatory neurotransmitter, an assumption which is in disagreement with the fact that the main observed effect of habenulo-raphe pathway stimulation is depression of 5-HT neuron firing (72–88% of the 5-HT neurons are inhibited; Stern et al., 1981). However, when a high stimulation frequency (15 Hz) is used, the habenulo-raphe pathway induces a marked increase in 5-HT release in projection areas, an effect that is blocked by injection of kynurenic acid into the DRN (Kalen et al., 1989). Such an observation has led Kalen et al. (1989) to suggest that EAAs are the main neurotransmitters in the habenulo-raphe pathway, and that glutamatergic fibers could have a double effect on 5-HT neuron, i.e., direct phasic stimulation and indirect inhibition by stimulating GABAergic interneurons. Such an observation is in keeping with in vitro findings in which stimulation of midbrain slices with an electrode in the DRN causes fast IPSPs and EPSPs that are respectively blocked by bicuculline or picrotoxin and the NMDA antagonists CNQX and APV (Pan et al., 1989; Pinnock 1992). The EPSPs due to electrically evoked release of EAA from afferent fibers onto 5-HT neurons may be reduced by activation of presynaptic inhibitory κ-opioid receptors located on the glutamatergic fibers (Pinnock, 1992). However, the predominant effect of systemically administered morphine is a stimulation of 5-HT release, an effect contrary to the one expected from activating inhibitory receptors on excitatory fibers impinging onto 5-HT neurons. A possible explanation for such a contradiction could be that the effect of activation of κ-receptors on EAA terminals may be overcome by activation of other opioid receptors, probably also located within the raphe nucleus, as suggested by Tao and Auerbach (1994). Apart from eliciting postsynaptic potentials on 5-HT neurons recorded from midbrain raphe slices, GABA and glutamate have been shown to modulate 5-HT release in rostral rhombencephalic raphe cells in primary cultures (Becquet et al., 1993b). GABA produces its negative modulation predominantly via $GABA_A$ but also $GABA_B$ receptors, whereas EAA induce 5-HT release by stimulating NMDA receptors (Becquet et al., 1993a). Furthermore, in vivo application of the $GABA_A$ antagonist picrotoxin into the DRN locally increased 5-HT release in unanesthetized rats (Becquet et al., 1990), suggesting that the latter receptors may induce a tonic inhibition of 5-HT release in the dorsal raphe. The release of 5-HT in the DRN is also modulated by tachykinins and catecholamines. Indeed, Substance P has been shown to increase 5-HT release in vitro in midbrain raphe slices (Kerwin and Pycock, 1979) and in vivo following its intraraphe injection (Reisine et al., 1982). The local infusion of amphetamine, apomorphine, and the selective D_2 receptor agonist quinpirole also increases 5-HT release in DRN, the effect of apomorphine being blocked by the selective D_2 receptor antagonist raclopride but not the D_1 antagonist SCH 23390 (Ferré and Artigas, 1993; Ferré et al., 1994). The latter observations confirm the role of D_2 receptors in modulating extracellular availability of 5-HT in the DRN, but whether the source of DA are dopaminergic afferents or DA neurons within the nucleus is still unknown. In the case of NA, this catecholamine inhibited K^+-evoked release of [^{3}H]5-HT from raphe slices. This effect was mimicked by α_2 adrenoceptor agonists clonidine and oxymetazoline, but not by the α_1 adrenoceptor agonists phenylephrine and methoxamine. Furthermore, yohimbine and rauwolscine not only blocked the effect of clonidine, but, when administered alone, they both increased the K^+-induced release of [^{3}H]5-HT. A possible interpretation of the auto- and heteroregulatory connections of 5-HT release in the DRN is given in Fig. 3.

Interestingly, in some cases, heteroregulatory influences may produce their effects via activation of autoregulatory mechanisms, e.g., D_2 receptor stimulation induces an increase in DRN 5-HT release and a decrease in striatal release, the latter effect being blocked by the administration of (+)-WAY 100135 (Ferré et al., 1994).

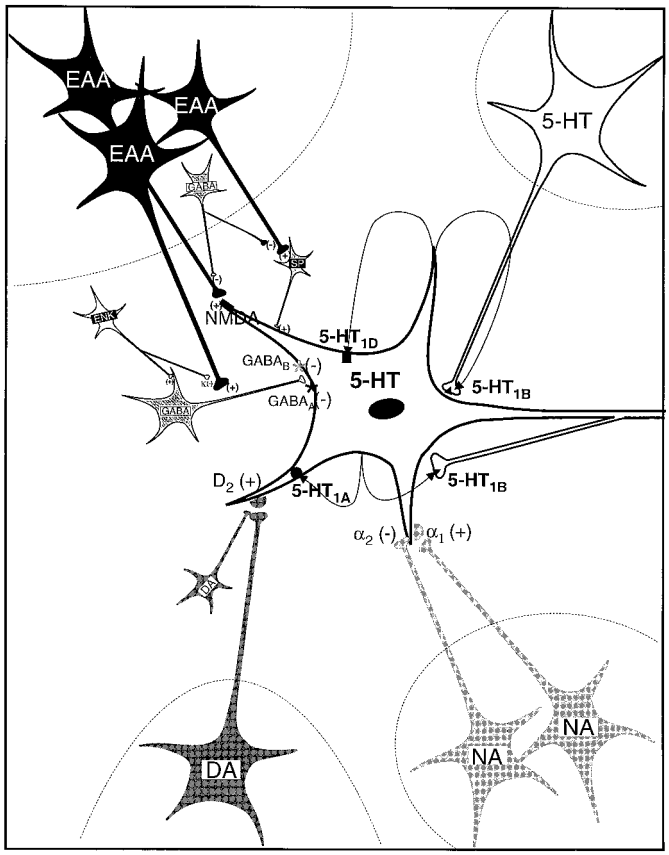

FIG. 3. Graphic representation of auto- and heteroregulatory connections of midbrain 5-HT neurons: somatodendritic 5-HT$_{1A}$ as well as 5-HT$_{1D/1D\text{-like}}$ receptors are principally influenced by extracellular 5-HT of somatodendritic origin. They respectively inhibit 5-HT neuron firing or local release of neurotransmitter. Extracellular 5-HT also modulates terminal 5-HT$_{1B}$ autoreceptors located either on axon collaterals from efferent axons of local 5-HT neurons or those located on axons impinging from neighboring 5-HT nuclei. Activation of α_1 adrenoceptors increases 5-HT neuron firing activity and that of α_2 adrenoceptors inhibits 5-HT release. Both receptors are stimulated by NA released from axons originating in locus ceruleus. Pharmacological stimulation of dopaminergic D$_2$ receptors increases local 5-HT release. It is not clear whether these receptors are physiologically stimulated by DA released from intraraphe DA neurons or dopaminergic projections originating in the VTA. The stimulation of the habenulo-raphe pathway is believed to induce glutamate release, which, via NMDA receptors, evokes a direct EPSP on 5-HT neurons. Glutamate also stimulates interneurons which may contain either Substance P or GABA. Although GABA inhibits 5-HT neuron firing activity (mainly via GABA$_A$ receptors), Substance P may produce an increase in local 5-HT release. Finally, GABAergic and enkephalinergic interneurons both inhibit glutamate release. Dashed lines, afferent nuclei boundaries.

In other cases, heteroregulatory mechanisms may override autoregulation, e.g., local infusion of Substance P enhances 5-HT release in the dorsal raphe (Reisine et al., 1982) and in the hippocampus (Gradin et al., 1992), or the contrary is observed with GABA which, in spite of decreasing somatodendritic availability of 5-HT when injected into the DRN, also decreases striatal 5-HT release (Becquet et al., 1990).

5. Autoregulation of 5-HT Release in Terminal Projection Areas: Cortex and Hippocampus. Evidence indicating that activation of 5-HT$_{1B}$ terminal autoreceptors inhibit 5-HT release is extensive and convincing (rat, mouse, guinea pig, pig, rabbit, human brain; see Table 4 for pharmacological profiles of terminal autoreceptors; Hoyer and Middlemiss, 1989; Starke et al., 1989; Göthert, 1990; Middlemiss and Hutson, 1990). Apart from this well established fact, there is now considerable evidence indicating that more than one receptor subtype could be involved in controlling release in the same region, and that some of these receptors might not be of the 5-HT$_{1B}$ subtype. In rat cortical slices, 8-OH-DPAT has been shown to inhibit electrically or K^+-evoked 5-HT release (Hamon et al., 1984; Limberger et al., 1991). Because such an effect was not blocked by 300 nM of the 5-HT$_{1B}$ antagonist isamoltane (Waldmeier et al., 1988; Schoeffter and Hoyer, 1989), it was suggested that 5-HT$_{1D}$ isamoltane-resistant sites might contribute to regulate 5-HT release in the rat frontal cortex (Limberger et al., 1991). Today we know that 8-OH-DPAT has considerable affinity not only for 5-HT$_{1D}$, but also 5-HT$_5$ and 5-HT$_7$ receptors (Table 5), and also that mRNAs for all three receptor subtypes are present in the DRN (Table 5). This suggests that, similar to the 5-HT$_{1B}$ receptor subtype, 5-HT$_{1D}$, 5-HT$_5$, as well as 5-HT$_7$ receptors could be expressed as autoreceptors on 5-HT neurons. The concentrations at which 8-OH-DPAT effectively inhibited evoked 5-HT release in the above-mentioned studies were higher than 100 nM. Thus, it is tempting to speculate that the probability for 5-HT$_{1D}$ or 5-HT$_5$ receptors to be involved in this effect is greater than for 5-HT$_7$ receptors, since the affinity for the latter is very high (Table 5) and 8-OH-DPAT would have been expected to be effective at lower concentrations. Moreover, it should be noted that at concentrations of up to 1 μM 8-OH-DPAT, some groups were unable to demonstrate an inhibition of cortical evoked release of 5-HT (Middlemiss, 1984b; Engel et al., 1986; Maura et al., 1986). Additional evidence supporting the fact that 5-HT receptors controlling 5-HT release in rat hippocampus are heterogeneous has been obtained by our group. In superfusion experiments, sumatriptan (1–1000 nM) and CP 93129 (1–300 nM) induced a dose-dependent inhibition of electrically evoked [^{3}H]5-HT release from rat hippocampal slices. Although the effects of CP 93129 and sumatriptan were blocked by (±)-cyanopindolol (1 μM), only that of sumatriptan was blocked by mianserin (0.3 and 1 μM). On the other hand, only the effect of CP 93129 was blocked by (−)-propranolol (0.3 μM). Moreover, incubation of rat hippocampal slices with the alkylating agent *N*-ethylmaleimide (NEM), abolished the effect of CP 93129 but not that of sumatriptan (Piñeyro and Blier, 1996). Furthermore, the inhibitory effect of 5-MeOT in rat hippocampus has been shown to remain unaffected by NEM, pertussis, or cholera toxin pretreatment, further indicating that, in the rat, there is an hippocampal 5-HT receptor subpopulation which is indeed resistant to G$_{i/o}$ protein inactivation (Blier, 1991). Results from superfusion studies in 5-HT$_{1B}$ knockout mice provide further evidence that a nonhomogeneous terminal autoreceptor population exists: non-5-HT$_{1B}$ re-

ceptors continue to control 5-HT release in terminal projection areas in 5-HT_{1B} knockout mice (Piñeyro et al., 1995a). Moreover, it is possible that the combination of receptor subtypes in terminal autoreceptor populations may differ among specific projection areas (e.g., cortex and hippocampus). This would not be surprising given that each of the 5-HT nuclei contribute in a different manner to the innervation of distinct terminal fields. In vivo experiments in the frontal cortex and hippocampus of 5-HT_{1B} knockout mice also support the idea that non-5-HT_{1D}, non-5-HT_{1B} receptors regulate 5-HT release in the mouse frontal cortex and that terminal 5-HT autoreceptor populations in the cortex and hippocampus may be different (Trillat et al., 1996).

In nonrodent species, an inhibitory effect of 8-OH-DPAT on terminal 5-HT release (Feuerstein et al., 1987; Schlicker et al., 1989) is not surprising given the moderate affinity of the drug for the nonrodent 5-HT_{1B} receptor (Table 5). However, until more selective ligands become available, a role for 5-HT_5, 5-HT_7 receptors, or even other 5-HT receptors not as yet characterized, on terminal 5-HT release may not be ruled out. Methiothepin is the only antagonist that effectively blocks the inhibition induced by 8-OH-DPAT (Feuerstein et al., 1987), but, since methiothepin binds with high affinity to all 5-HT_{1D}, 5-HT_5, and 5-HT_7 receptor subtypes, no further inferences can be made. In the guinea pig hippocampus, but not in the cortex, methiothepin has been shown to block the inhibitory effect of 5-CT and sumatriptan with less potency than that of 5-HT (Wilkinson and Middlemiss, 1992; Wilkinson et al., 1993), suggesting an heterogeneity in the receptor subtypes regulating 5-HT release in the former but not the latter region, where methiothepin equipotently blocks the effects of both 5-HT and sumatriptan. Differences among 5-HT receptor populations controlling 5-HT release in the cortex and hippocampus in the guinea pig brain has also been reported by El Mansari and Blier (1996). These authors showed that G protein inactivation with NEM attenuates the inhibitory effect of 5-MeOT in the cortex, but not in the hippocampus, although the inhibitory effect of sumatriptan was reduced in NEM-pretreated slices from both regions. The difference between cortical and hippocampal 5-HT autoreceptor populations is further supported by the fact that the inhibitory effect of 5-MeOT on electrically evoked release of [^{3}H]5-HT is attenuated in hippocampus but not frontal cortex slices obtained from guinea pigs treated with paroxetine for 21 days (Blier and Bouchard, 1994; El Mansari et al., 1995).

In vitro as well as in vivo studies have shown that the amount of 5-HT released per electrical impulse increases with decreasing frequencies of stimulation (Göthert 1980; Baumann and Waldmeier, 1981; Chaput et al., 1986a; Blier et al., 1989a), probably due to a lower degree of activation of autoreceptors by prolonging the interval between the stimulation pulses. This interpretation is supported by findings indicating that, at higher stimulation frequencies, the effectiveness of terminal 5-HT receptor agonists (5-HT itself, 5-CT, 5-MeOT) to inhibit 5-HT release is reduced and that of the antagonist methiothepin to enhance this parameter is increased (Baumann and Waldmeier, 1981; Chaput et al., 1986a; Blier et al., 1989a). Another way in which the concentration of endogenous 5-HT in the biophase of 5-HT autoreceptors may be increased is by inhibition of neuronal 5-HT reuptake. Similar to high stimulation frequencies, in the presence of 5-HT reuptake blockers, the effectiveness of 5-HT agonists is reduced (Langer and Moret, 1982; Galzin et al., 1985) and that of antagonists is enhanced (Feuerstein et al., 1987). To explain SSRI-induced changes in efficacy, two alternative interpretations have been proposed: 1) an increase in 5-HT concentration in the biophase of terminal autoreceptors, and 2) an interaction between neuronal 5-HTT and terminal 5-HT receptors (Langer and Moret, 1982; Galzin et al., 1985; Passarelli et al., 1987; Moret and Briley, 1988). Two important arguments against the "molecular link hypothesis" favor the "5-HT biophase hypothesis": 1) in experiments on synaptosomes in which the released transmitter is washed away rapidly and 5-HT in the biophase remains too low for autoreceptor activation, and 5-HT receptor agonists produce the same inhibition of synaptosomal [^{3}H]5-HT release in the absence or presence of reuptake blockers (Raiteri et al., 1984; Bonanno and Raiteri, 1987), and 2) when very short trains of high frequency pulses are used in brain slices, release of [^{3}H]5-HT is measurable, and yet pulses are too short to generate autoinhibition (Limberger et al., 1991). In the latter circumstances, 5-CT and 5-MeOT generate similar concentration-effect curves for the inhibition of evoked [^{3}H]5-HT release in the absence and presence of different reuptake blockers (Limberger et al., 1990). On the other hand, the fact that 5-HT reuptake blockers continue to reduce the effectiveness of 5-HT autoreceptor agonists following 5-HT depletion by the 5-HT synthesis inhibitor PCPA has been used as an additional argument to support the molecular link hypothesis (Galzin et al., 1985; Passarelli et al., 1987). It is important to notice, however, that even after a 90% depletion of hippocampal 5-HT by PCPA pretreatment, 5-HT pre- and postsynaptic functions were found to be unchanged (Chaput et al., 1990), suggesting that the 5-HT system maintains its adaptability even after a great reduction of endogenous 5-HT.

The effect of changes in 5-HT biophase concentration are particularly evident in the case of partial agonists which can either inhibit or enhance terminal 5-HT release depending on the circumstances. For example, in the absence of the SSRI nitroquipazine or fluoxetine (1 μM), the 5-HT_{1B} ligand (±)-cyanopindolol inhibited electrically evoked release of 5-HT in the rat hippocampus and enhanced the same parameter when the slices were superfused with the uptake inhibitors (Feuerstein et al.,

1987). Similarly, in conditions of negligible autoinhibition (4 pulses at 100 Hz), metergoline inhibited [^{3}H]5-HT release from rabbit frontal cortex slices (Limberger et al., 1991), had no effect when 5-HT biophase was higher (360 pulses at 3 Hz), and enhanced release when 360 pulses at 3 Hz were delivered in the presence of reuptake inhibitors (Feuerstein et al., 1987). Table 6 summarizes the effects on evoked 5-HT release in the cortex and hippocampus, obtained with different terminal autoreceptor antagonists in different species and using different stimulation paradigms. Methiothepin has been shown to consistently enhance evoked release of 5-HT in conditions where autoinhibition exists, suggesting that it might be a pure antagonist. Furthermore, under the same conditions (360 pulses at 3 Hz; rat hypothalamic slices) in which antagonists such as metergoline and alprenolol had no effect by themselves but blocked the effect of LSD, methiothepin induced opposite effects to those of the terminal autoreceptor agonist. This observation has prompted the suggestion that methiothepin could possess inverse agonistic properties at the terminal 5-HT autoreceptor (Moret and Briley, 1993). More recently, this suggestion has been proved, because methiothepin was found to increase [^{35}S]GTPγS binding to human 5-HT$_{1D}$ and 5-HT$_{1B}$ receptors (Jones et al., 1995), behaving as an inverse agonist. This observation suggests that methiothepin binding to 5-HT$_{1B/1D}$ receptors favors the uncoupling of the receptor-G protein complex. An inverse agonist at the human terminal autoreceptor that would enhance 5-HT release in terminal projection areas might prove an effective antidepressant with quick onset of action.

5-HT release in guinea pig and rat cortex and hippocampus is also modulated by 5-HT$_3$ receptors (Galzin et al., 1990; Barnes et al., 1992; Martin et al., 1992; Blier and Bouchard, 1993). Unlike 5-HT$_{1B/1D}$, 5-HT$_3$ receptors are not localized on 5-HT terminals (Blier et al., 1993c); they enhance 5-HT release (Galzin et al., 1990; Barnes et al., 1992; Martin et al., 1992; Blier and Bouchard, 1993) and desensitize within minutes of agonist exposure (see Hoyer 1990; Blier and Bouchard, 1993). They share with terminal autoreceptors their frequency dependence, being more effective at enhancing 5-HT release at lower than at higher frequencies (Blier and Bouchard, 1993).

6. Heteroregulation of Neurotransmitter Release from 5-HT Fibers in Cortex and Hippocampus. Several in vivo and in vitro studies using brain slices or synaptosomes have provided evidence for the involvement of multiple neurotransmitters in the local regulation of 5-HT release in terminal projection areas. α_2-Adrenergic heteroreceptors on 5-HT terminals in the brain of different species have long been known to inhibit 5-HT release (Gobbi et al., 1990; Raiteri et al., 1990; see Göthert and Schlicker, 1991; Maura et al., 1992b; Blier et al., 1993c). Several findings support the idea that the α_2-adrenergic heteroreceptors have different properties as compared with α_2 autoreceptors regulating NA release in terminal projection areas: 1) they have different pharmacological profiles (Raiteri et al., 1983b; Maura et al., 1992b; Mongeau et al., 1993), 2) α_2 auto- and heteroreceptors are differentially modulated by neuropeptides such as NPY (Martire et al., 1989), and 3) only the former desensitize following long-term treatment with befloxatone, a selective MAO-A inhibitor (see Blier et al., 1993c). The idea of distinct functional properties of adrenoceptors regulating 5-HT and NE release in terminal projection areas has been exploited in the development of new antidepressant drugs. Interestingly, selective α_2 heteroreceptor antagonists, even given acutely, may have the capacity to enhance 5-HT neurotransmission (Haddjeri et al., 1996). Like NE, histamine also exerts a negative regulation of 5-HT release, probably via activation of H$_3$ heteroreceptors (Fink et al., 1990). On the other hand, acetylcholine (ACh) enhances forebrain 5-HT release by activating nicotinic receptors (Toth et al., 1992; Summers and Giacobini, 1995). The fact that kynurenic acid almost completely prevents the enhancing effect on neurotransmitter release induced by the local administration of nicotine suggests that its effect on 5-HT release is indirect, mediated via glutamic acid release (Toth et al., 1992). Inhibitory amino acids are also involved in the regulation of 5-HT release in cortex and hippocampus. Following systemic administration of benzodiazepine agonists, spontaneous or evoked release of 5-HT in either region has been shown to decrease (Hitchcott et al., 1990; Broderick, 1991; Cheng et al., 1993). Local effects are more ambiguous. In the hippocampal formation, for example, the activation of the GABA$_A$ receptor complex has been shown to produce either a decrease, no effect, or an increase in 5-HT release (Pei et al., 1989; Lista et al., 1990). The fact that the local application of the Cl^- channel blocker picrotoxin induced a robust increase in hippocampal 5-HT of freely moving rats supports the idea that GABA exerts a tonic inhibition of 5-HT release in this region (Pei et al., 1989). Numerous polypeptides have also been found to locally modulate 5-HT release in projection areas. For example, PYY and pancreatic polypeptide inhibit cortical 5-HT release via the activation of the same presynaptic G protein-coupled receptor as NPY (Schlicker et al., 1991). On the other hand, Substance P and neurokinin A, two coexisting neuropeptides of the tachykinin family, stimulate 5-HT release in this same area (Iverfeldt et al., 1990). Pharmacological activation of local opioid receptors (δ, κ, and μ) suggests that endogenous hippocampal opiates may also be involved in the negative regulation of 5-HT release in this area (Passarelli and Costa, 1989; Cui et al., 1994).

7. 5-HT$_{1B}$ versus 5-HT$_{1D}$ Receptors. 5-HT$_{1B}$-binding sites, as opposed to 5-HT$_{1A}$ sites, were initially pharmacologically described. They represented the sites that bind spiperone and 8-OH-DPAT with low affinity (Pedigo et al., 1981; Middlemiss and Fozard, 1983) but present high affinity for ^{125}I-cyanopindolol (Hoyer et al.,

1985a,b). They have also been defined by exclusion as [^{3}H]5-HT-binding sites which are neither of the 5-HT$_{1A}$ nor of the 5-HT$_{2C}$ subtype (Blurton and Wood, 1986; Peroutka, 1986; Alexander and Wood, 1987). Originally, these pharmacologically described 5-HT$_{1B}$ sites were described in rodents (Hoyer et al., 1985a; Waeber et al., 1989a; Waeber and Palacios, 1992) but not in guinea pig, pig, cow, or human brain (Heuring et al., 1986; see Bruinvels et al., 1993 and references within). In nonrodent species, the sites visualized in the presence of saturating concentrations of 8-OHDPAT and mesulergine (Heuring and Peroutka, 1987; Waeber et al., 1988, 1989b) were originally named 5-HT$_{1D}$ and more recently reclassified as 5-HT$_{1B}$. Hence, in spite of their pharmacological differences, sites defined by exclusion in rodent and nonrodent brain would fall under the present 5-HT$_{1B}$ category. However, it has been established that the sites labeled in this way represent an heterogeneous receptor population composed of at least 5-HT$_{1B/1D}$ (5-CT-sensitive) and 5-HT$_{1E}$ (5-CT-insensitive) receptors (Leonhardt et al., 1989; Sumner and Humphrey, 1989; Beer et al., 1992; Lowther et al., 1992; Miller and Teitler, 1992). Furthermore, [^{3}H]5-CT has been shown to label two different subpopulations of 5-HT$_1$ as high-affinity sites in guinea pig cortex and striatum (Mahle et al., 1991). It was the development of an iodinated radioligand, serotonin-5-*O*-carboxymethyl-glycyl-^{125}I-tyrosinamide (^{125}I-GTI; Boulenguez et al., 1991, 1992), that allowed 5-HT$_{1B}$ binding sites to be directly labeled in human, nonhuman primate, and guinea pig brain, among others (Bruinvels et al., 1991, 1992).

Rodent versus nonrodent 5-HT$_{1B}$ receptor subtype pharmacology is quite different, whereas that of 5-HT$_{1D}$ and nonrodent 5-HT$_{1B}$-binding sites is very much alike. Two pharmacologically distinct groups may be thus defined, rodent 5-HT$_{1B}$ and nonrodent 5-HT$_{1B}$ and all species 5-HT$_{1D}$ receptors. Compounds that show at least a 1.5 log-unit difference in their affinities for rodent 5-HT$_{1B}$ versus nonrodent 5-HT$_{1B}$/5-HT$_{1D}$-binding sites in mammalian brain membranes are summarized in Table 7.

Recently, human 5-HT$_{1B/1D}$ (Hamblin and Metcalf, 1991; Adham et al., 1992; Demchyshyn et al., 1992; Hamblin et al., 1992b; Jin et al., 1992; Levy et al., 1992; Weinshank et al., 1992) and rat 5-HT$_{1B}$ receptors (Voigt et al., 1991; Adham et al., 1992; Hamblin et al., 1992a; Maroteaux et al., 1992) have been cloned. From these studies, it has become clear that the "old" 5-HT$_{1D}$ pharmacological phenotype is conferred by two separate genes, i.e., a *5-HT$_{1B}$* gene cloned in nonrodents (Adham et al., 1992; Demchyshyn et al., 1992; Hamblin et al., 1992b; Jin et al., 1992; Levy et al., 1992; Weinshank et al., 1992) and a *5-HT$_{1D}$* gene cloned in rodents and nonrodents (Hamblin et al., 1992a; Weinshank et al., 1992). Although the overall amino acid similarity between 5-HT$_{1D}$ and nonrodent 5-HT$_{1B}$ receptors, which share an almost identical pharmacology, is 61 to 63% (Hamblin et al., 1992b; Weinshank et al., 1992), nonrodent 5-HT$_{1B}$ and rat 5-HT$_{1B}$ receptors share a 93% similarity in their deduced amino acid sequence and yet have different pharmacological profiles. When expressed in heterologous systems, nonrodent 5-HT$_{1B}$/5-HT$_{1D}$ and rodent 5-HT$_{1B}$ receptors maintain the same pharmacological profile as in brain membranes: the former have a higher affinity for sumatriptan, 8-OH-DPAT, and α_2-adrenergic antagonists, and bind CP 93129 and arylalkylamine compounds such as propranolol and pindolol with lower affinity than the rodent 5-HT$_{1B}$ subtype (Metcalf et al., 1992; Oksenberg et al., 1992; Parker et al., 1993; Adham et al., 1994). Interestingly, a single amino acid difference (asparagine versus threonine at position 355) has been shown to be responsible for rodent versus nonrodent distinct pharmacological profiles. Their pharmacological phenotypes may be interconverted by a single point mutation at amino acid 355 (Metcalf et al., 1992; Oksenberg et al., 1992; Parker et al., 1993; Adham et al., 1994). The relationship between 5-HT$_{1D}$, nonrodent 5-HT$_{1B}$ and rodent 5-HT$_{1B}$ receptors

TABLE 7
Compounds that allow a pharmacological distinction between 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors

Compound	Log Difference	Reference
Compounds with higher affinity for rodent 5-HT$_{1B}$ than 5-HT$_{1D}$/non-rodent 5-HT$_{1B}$ receptors (difference in affinity stated in log units)		
(−)-SDZ 21009	>3	Hoyer et al., 1985; Hoyer and Schoeffter, 1991
CP93129	>2	Bruinvels et al., 1991; Koe et al., 1992
(−)-Pindolol	>1.9	Hoyer et al., 1985; Heuring et al., 1987; Hoyer and Schoeftter, 1991
Cyanopindolol	>1.8	Engel et al., 1986; Hoyer and Schoeffter 1991
(−)-Propranolol	>1.8	Hoyer et al., 1985; Heuring et al., 1987; Hoyer and Schoeffter, 1991
Compounds with higher affinity for 5-HT$_{1D}$/non-rodent 5-HT$_{1B}$ than rodent 5-HT$_{1B}$ receptors (difference in affinity stated in log units)		
Yohimbine	>1.8	Heuring et al., 1987; Bruinvels et al., 1991; Hoyer and Schoeffter, 1991
Sumatriptan	>1.5	Van Wijngaarden et al., 1990; Bruinvels et al., 1991
Mianserin	>1.5	Hoyer et al., 1985; Peroutka et al., 1986; Heuring et al., 1987; Bruinvels et al., 1991; Hoyer and Schoeffter, 1991
8-OH-DPAT	>1.5	Heuring et al., 1987; Van Wijngaarden et al., 1990; Bruinvels et al., 1991; Hoyer and Schoeffter, 1991

has been summarized by Hartig et al. (1992): given the 70 to 80% similarity in their transmembrane domains, human 5-$HT_{1B/1D}$ and rodent 5-$HT_{1B/1D}$ receptors should be considered pairs of intraspecies receptor subtypes and members of the same gene product subfamily. Furthermore, given the high (95%) transmembrane homology between human 5-HT_{1D} and rat 5-HT_{1D} receptors, on the one hand, and corresponding 5-HT_{1B} receptors on the other, these two receptor pairs should be considered as species homologs. It was only recently that the 5-HT_2 receptor antagonists ritanserin and ketanserin were found to discriminate between 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (1.1–1.8 log-unit difference in K_D values, 5-HT_{1D} receptors having a higher affinity than nonrodent 5-HT_{1B} receptors for the 5-HT_2 antagonists (Doménech et al., 1994; Zgombick et al., 1995). Before, these receptors were initially thought to be pharmacologically indistinguishable. Although less selective, GR 127935 and metergoline also distinguish between the two receptors with higher affinity for the nonrodent 5-HT_{1B} subtype (1 and 0.6 log units, respectively; Skingle et al., 1991; Doménech et al., 1994). Moreover, in transected cells, GR 127935 further distinguishes 5-HT_{1D} from nonrodent 5-HT_{1B} receptors by eliciting a full agonistic response for the former and behaving as a silent antagonist for the latter (Pauwels and Colpaert, 1995; Pauwels and Palmier, 1995). However, although transfected systems constitute a useful first approach for studying receptor pharmacology, interpretation of functional data from these studies should be cautious since drug activity may vary according to the level of receptor expression. For example, the arylkylamines propranolol and pindolol, and the ergot derivative metergoline, which have been described as antagonists or partial agonists in in vivo or in vitro studies of terminal 5-HT autoreceptors, were found to behave as full agonists in cells expressing the rat or human 5-HT_{1B} receptor (Miller et al., 1992; Adham et al., 1993b). Hence, further development of selective 5-HT_{1D}/nonrodent 5-HT_{1B} compounds will depend on the availability of naturally occurring systems expressing the different receptors to allow drug evaluation. Hamel and coworkers have demonstrated that nonrodent 5-HT_{1B} receptors mediate 5-HT-induced contractions in cerebral arteries, establishing this tissue as an appropriate model of nonrodent 5-HT_{1B} receptor function (Hamel and Bouchard, 1991; Hamel et al., 1993). On the other hand, the low expression of 5-HT_{1D} relative to 5-HT_{1B} receptors in mammalian brain (Beer and Middlemiss, 1993; Bruinvels et al., 1993, 1994b; Doménech et al., 1994) has precluded identification of potentially 5-HT_{1D}-selective compounds using native systems.

The use of autoradiographic and in situ hybridization techniques has allowed us to compare not only the anatomical but also the cellular distribution of 5-$HT_{1B/1D}$-binding sites and 5-$HT_{1B/1D}$ mRNAs (Bruinvels et al., 1994a; Doucet et al., 1995). Autoradiographic studies of ^{125}I-cyanopindolol (Pazos et al., 1985) and, more recently, ^{125}I-GTI (Bruinvels et al., 1993) have revealed a particularly high concentration of 5-HT_{1B}-binding sites in striatum, substantia nigra, and dorsal subiculum of the rat brain. Yet no mRNA for 5-HT_{1B} receptors was found in the two latter regions (Voigt et al., 1991; Bruinvels et al., 1993; Doucet et al., 1995). Conversely, the DRN, which exhibited an intense mRNA hybridization signal, displayed low or no 5-HT_{1B} binding (Vergé et al., 1986; Voigt et al., 1991; Bruinvels et al., 1993; Doucet et al., 1995). Mismatches between 5-HT receptor protein and mRNA previously observed in mice have been explained by assuming that 5-HT_{1B} receptors are transported along fibers far from somas where they are synthesized (Boschert et al., 1994). For example, it has been suggested that 5-HT_{1B} receptors synthesized in the soma of CA_1 pyramidal neurons (CA_1 pyramidal neurons are intensely labeled for mRNA; Doucet et al., 1995) are transported via their glutamatergic projections to reach the dorsal subiculum as heteroreceptors. Similarly, 5-HT_{1B} heteroreceptors on striatal projections to the substantia nigra have been proposed to explain the high binding and low hybridization observed in this region (Bruinvels et al., 1994a). Moreover, 5-HT_{1B} receptors synthesized in the midbrain raphe could be transported via axons to reach terminal areas where they have been described as autoreceptors.

Autoradiographic studies on the human brain indicate that very high densities of 5-$HT_{1B/1D}$ receptors labeled by ^{125}I-GTI are present in the basal ganglia, and especially in substantia nigra (Bruinvels et al., 1991; Palacios et al., 1992). A similar population of ^{125}I-GTI-labeled sites has also been identified in human cortical membranes (Beer and Middlemiss, 1993) and guinea pig basal ganglia (Bruinvels et al., 1994b). In the rat brain, ^{125}I-GTI also labels 5-HT_{1B} as well as 5-HT_{1D} sites. The latter have been defined as the non-5-HT_{1B}-^{125}I-GTI binding displaced by the 4[2-[4-[3-(trifluoromethyl)phenyl]1-piperazinyl]ethyl]benzeneamine (PAPP, which has more than 2 log units higher affinity for 5-HT_{1D} than 5-HT_{1B} receptors; Schoeffter and Hoyer, 1989). These PAPP displaceable sites represent 15 and 11% of cortical and striatal iodine-labeled sites in the rat brain (Bruinvels et al., 1993). In the rat cortex, the percentage of total ^{125}I-GTI sites (5-HT_{1B} + 5-HT_{1D}) displaced by PAPP (designated 5-HT_{1D} sites; 15%) is in agreement with the percentage of total ^{125}I-GTI (5-HT_{1B} + 5-HT_{1D}) minus ^{125}I-cytochrome P-450 (CYP; 5-HT_{1B}) in the same region (19%) and with the percentage of total 5-HT_1 binding represented by non-5-$HT_{1A/1B/2C}$ (5-HT_{1D}) cortical sites reported by Herrick-Davis and Titeler (18%; 1988). In contrast, in the striatum, the sites defined by ^{125}I-GTI (5-HT_{1B} + 5-HT_{1D}) minus ^{125}I-CYP (5-HT_{1B}) and non-5-$HT_{1A/1B/2C}$ (5-HT_{1D}) sites were 20 and 30%, respectively (Herrick-Davis and Titeler, 1988; Bruinvels et al., 1993), representing more than double the PAPP displacement sites (11%) in this region. In the same study, Herrick-Davis and Titeler (1988) reported more

than 40% of non-5-$HT_{1A/1B/2C}$ sites with 5-HT_{1D} receptor pharmacology (5-HT > 5-CT ≫ TFMPP > 8-OH-DPAT ≫ (±)-pindolol) in midbrain membranes. On the other hand, Bruinvels et al. (1993) reported an average of 8% PAPP-defined 5-HT_{1D} sites in the same area, whereas the 5-HT_{1B} sites labeled by ^{125}I-CYP in the dorsal raphe and central gray represented approximately 60% of the 5-$HT_{1B/1D}$ sites labeled by ^{125}I-GTI). These observations may indicate that PAPP does not identify all of the 5-HT_{1D} sites or that ^{125}I-GTI labels other non-5-$HT_{1B/1D}$ sites. Given that the non-5-$HT_{1A/1B/2C}$ population described by Herrick-Davis and Titeler (1988) showed high affinity for 5-CT, it is improbable for them to be 5-$HT_{1E/1F}$ sites. However, the presence of multiple receptors within this population cannot be ruled out, since Hill coefficients for sites that recognized 5-CT ranged from 0.62 to 0.86 (Herrick-Davis and Titeler, 1988). Hence, the above-mentioned observations suggest that ^{125}I-GTI binding displaced by PAPP may not represent all of the 5-HT_{1D} receptors in the rat raphe area. Rodent midbrain, especially raphe nuclei, is one of the areas expressing the highest levels of 5-HT_{1D} mRNA (Hamblin et al., 1992a; Bruinvels et al., 1994a). Thus, the high coexpression of 5-HT_{1D} sites (according to Herrick-Davis and Titeler, 1988) and mRNA could be interpreted as further support for the role of 5-$HT_{1D\alpha}$ as somatodendritic autoreceptors in the rat brain. Conversely, results from Bruinvels and coworkers (1993, 1994a,b) would support the idea that most of the 5-HT_{1D} or its mRNA are transported to terminal areas. On the other hand, there is evidence indicating a matching distribution of 5-HT_{1B} sites and mRNA in the rat midbrain; Bruinvels et al., 1993, 1994b; Doucet et al., 1995). However, when tested in functional studies, at concentrations that do not alter basal outflow, the 5-HT_{1B} agonist CP 93129 did not modify 5-HT release (Piñeyro et al., 1995b). As previously stated, a possible interpretation for this set of observations would be that 5-HT_{1B} receptors located on afferent or recurrent axons and regulating 5-HT release from these secondary sources (as compared to somatodendritic release) account for the presence of the protein, whereas mRNA would be mainly directed to the synthesis of 5-HT_{1B} autoreceptors in terminal projection areas.

D. Effect of Antidepressant Drug Administration on 5-HT Release

1. Administration of 5-HT Reuptake Blockers. The reported effect of acute 5-HT reuptake blockade depends on the dose used and the region examined. At high doses (10 mg/kg i.p or s.c), the acute administration of fluoxetine, citalopram, or sertraline has been shown to induce an increase in extracellular 5-HT in terminal projection areas such as cortex, striatum, or diencephalon (Dailey et al., 1992; Invernizzi et al., 1992a; Perry and Fuller, 1992; Rutter and Auerbach, 1993, see Fuller, 1994). The increase in extracellular 5-HT is dependent on neuronal firing because it is blocked by TTX (Perry and Fuller, 1992) or 8-OH-DPAT (Rutter and Auerbach, 1993). The latter observation is somewhat puzzling given the fact that ED_{50} of i.v. doses of different SSRIs to inhibit 5-HT neuron firing are within the 0.1 and 0.5 mg/kg range (Blier and de Montigny, 1980; Blier et al., 1984; Chaput et al., 1986b; Gartside et al., 1995; Hajós et al., 1995; Maudhuit et al., 1995; Kasamo et al., 1996), suggesting that at 10 mg/kg, no matter what SSRI is given, the result would be total shut-down of 5-HT neuron firing activity. Even if rats are much more rapid metabolizers than humans, a dose of 10 mg/kg is 15 to 35 times the therapeutic dose. Hence, other studies have been performed using much lower doses of SSRIs. In such cases, the systemic administration of 1 mg/kg citalopram or 32 μmol/kg sertraline (Invernizzi et al., 1991, 1992a) produced no increase in extracellular 5-HT in cortical projection areas, and experiments in which extracellular 5-HT was simultaneously measured in cortex and raphe nuclei reveal that, following systemic administration of these reuptake inhibitors, there is a preferential increase in extracellular 5-HT in the raphe region (Adell and Artigas, 1991; Bel and Artigas, 1992). It is this increase in somatodendritic extracellular 5-HT that activates the powerful 5-HT_{1A} autoreceptor feedback loop leading 5-HT neurons to establish their own "ceiling" on the extent to which uptake inhibitors increase extracellular 5-HT in terminal projection areas. Reasoning that long-term reuptake blockade induces a desensitization of somatodendritic 5-HT_{1A} autoreceptors, Bel and Artigas (1993) proposed the possibility to overcome negative feedback and increase extracellular availability of 5-HT in terminal projection areas even when using low doses of SSRIs. Indeed, they treated rats with 1 mg/kg fluvoxamine (s.c.) for 2 weeks, and at the end of this time period the increase in extracellular concentration of 5-HT in frontal cortex of treated rats, still carrying the osmotic minipump, was similar to that observed following a 10-mg/kg acute i.v. dose (Bel and Artigas, 1992, 1993). These results have been confirmed in a study by Invernizzi et al. (1994), in which sustained treatment with citalopram (10 mg/kg/day for 14 days) facilitated the enhancing effect on terminal 5-HT release produced by a dose of 1 mg/kg of the SSRI administered 24 h after the end of the treatment. Moreover, this study also showed that the reducing effect of a systemic dose of 25 μg/kg 8-OH-DPAT on terminal 5-HT release was abolished following long-term citalopram administration, thus confirming a desensitization of 5-HT_{1A} autoreceptors (Invernizzi et al., 1994). On the other hand, Bosker et al. (1995a,b) reported that a 14- or 21-day treatment with oral or s.c. fluvoxamine (3 mg/kg or 6.5 mg/kg at study outset, respectively) did not produce desensitization of 5-HT_{1A} autoreceptors, facilitation of another dose of fluvoxamine, nor increase in hippocampal 5-HT output. If indeed 5-HT_{1A} autoreceptor desensitization may in part account for the antidepressant effect of prolonged SSRI administration, an acute reduction of the activa-

tion of the feedback mechanism should produce an effect similar to that of sustained SSRI treatment. This assumption has been verified in seven of eight placebo-controlled clinical trials showing that in depressed patients the combination of pindolol and an SSRI produced either a significant acceleration of the antidepressant response or a greater proportion of patients presenting a response at the end of the trial (Maes et al., 1996, 1999; Berman et al., 1997; Pérez et al., 1997; Tome et al., 1997; Zanardi et al., 1997, 1998; Bordet et al., 1998). These results are in agreement with results referred to in a previous section, in which blockade of 5-HT_{1A} autoreceptors was shown to increase extracellular availability of 5-HT in terminal projection areas after the acute administration of an SSRI.

Apart from inducing desensitization of somatodendritic 5-HT_{1A} autoreceptors, sustained paroxetine (10 mg/kg/day s.c. for 21 days) administration has been reported to decrease the effectiveness of 5-$HT_{1B/1D}$ receptor activation in reducing electrically evoked release of 5-HT from rat and guinea pig midbrain raphe slices (El Mansari and Blier, 1996; Piñeyro and Blier, 1996). This same treatment, as well as fluoxetine (5 mg/kg/day i.p. for 21 days), induced an increase in electrically evoked 5-HT release from raphe slices (O'Connor and Kruk, 1994; El Mansari and Blier, 1996; Piñeyro and Blier, 1996), indicating that 5-HT receptors which negatively control 5-HT release in the somatodendritic area are less sensitive to activation by the endogenous neurotransmitter after sustained 5-HT uptake inhibition. In contrast, Bel and Artigas (1993) found no increase in basal extracellular 5-HT levels in the dorsal raphe of rats treated with fluvoxamine (1 mg/kg/day s.c. for 21 days).

Terminal 5-$HT_{1B/1D}$ autoreceptors in different projection areas including hippocampus, hypothalamus, and orbito-frontal cortex have also been found to desensitize after sustained administration of SSRIs. Desensitization has been demonstrated by a reduced efficacy of 5-$HT_{1B/1D}$ agonists to inhibit evoked 5-HT release (Blier and de Montigny, 1983; Blier et al., 1984, 1998a,b; Chaput et al., 1986b; Moret and Briley, 1990; O'Connor and Kruk, 1994; Blier and Bouchard, 1994; El Mansari et al., 1995). Such a desensitization results in a greater release of 5-HT per action potential, as indicated by a greater inhibition of the firing activity of CA_3 pyramidal neurons following 5-HT pathway stimulation (in the absence of changes in postsynaptic receptor sensitivity; Blier and de Montigny, 1983; Blier et al., 1984, 1988a,b; Chaput et al., 1986b), as well as an increase in electrically evoked release of [^{3}H]5-HT from preloaded slices of different projection areas, following a 48-h washout period after SSRI administration (Blier and Bouchard, 1994; El Mansari et al., 1995). Interestingly, not all terminal regions respond in a similar way to the same SSRI treatment, e.g., after a 21-day treatment with paroxetine (10 mg/kg/day s.c.), the inhibitory effect of the agonist 5-methoxytryptamine on the evoked release of 5-HT was attenuated in slices of hippocampus and hypothalamus but not of frontal or orbito-frontal cortex (Blier and Bouchard, 1994, El Mansari et al., 1995). In turn, of the two latter regions, only in the orbito-frontal cortex did desensitization occur after an 8-week treatment with paroxetine (10 mg/kg/day s.c.; El Mansari et al., 1995). It is still unclear whether these different results indicate that 5-HT neurons are endowed with different autoreceptor populations depending on the terminal region to which they project, or whether it is the local influences that determine the different adaptative properties of the same autoreceptor subtype. Sustained administration of a low dose of fluvoxamine was found to induce desensitization of somatodendritic 5-HT_{1A} receptors but not of 5-$HT_{1B/1D}$ terminal autoreceptors, the sensitivity of which remained unchanged after fluoxetine (5 mg/kg/day for 21 days) or fluvoxamine (6.7 mg/kg/day for 28 days; Bel and Artigas, 1993; Bosker et al., 1995b; El Mansari et al., 1995) administration. Furthermore, chlomipramine (10 mg/kg/day s.c. for 21 days) did not produce a desensitization of 5-HT_{1B} terminal autoreceptors in rabbit hypothalamus (Schoups and De Potter, 1988), probably due to the fact that, although in vitro this drug is a potent and highly selective 5-HT reuptake blocker, in vivo it loses its selectivity as soon as it is degraded to chlordesipramine. It is thus possible that a high percentage or complete blockade of 5-HT reuptake sites should be achieved for terminal autoreceptor desensitization to occur.

The importance of terminal autoreceptor plasticity in ensuring an enhanced 5-HT transmission is further supported by the observation that prolonged administration of SSRIs reduces brain 5-HT (Hrdina, 1987; Caccia et al., 1992; Trouvin et al., 1993), indicating that after prolonged reuptake blockade, an increase in extracellular 5-HT availability occurs in the face of a reduction in the total 5-HT tissue content.

2. Prolonged Administration of MAOIs. In this case the situation is reversed, brain 5-HT being actually increased after sustained blockade of MAO-A (Blier et al., 1986a,b; Celada and Artigas, 1993; Ferrer and Artigas, 1994). On the other hand, similar to SSRIs, the acute systemic administration of selective MAO-A or nonselective MAO-A/MAO-B inhibitors produces an immediate inhibition of 5-HT metabolism and a reduction in 5-HT neuron firing activity (Blier and de Montigny, 1985; Blier et al., 1986a,b). It is not surprising then that, when given acutely, MAOIs and SSRIs, produce a preferential increase in extracellular 5-HT in midbrain raphe nuclei as compared to terminal projection areas. In contrast, prolonged MAOI administration increases extracellular availability of 5-HT to a similar extent in pre- and postsynaptic projection areas (Celada and Artigas, 1993; Ferrer and Artigas, 1994; Bel and Artigas, 1995) with a time course similar to that of the desensitization of somatodendritic 5-HT_{1A} autoreceptors (Blier and de Montigny, 1985; Blier et al., 1986a,b; Piñeyro and Blier,

1996). In long-term experiments, tranylcypromine given at a dose that had no effect in acute experiments (0.5 mg/kg/day s.c. for 14 days) produced a greater increase in extracellular cortical availability of 5-HT than the acute administration of a dose six times higher, even if the increases in tissue 5-HT concentrations were of 40 and 700%, following the long-term/low-dose and acute/high-dose treatments, respectively. These observations suggest that even if the increase in intracellular 5-HT is almost 20-fold higher following an acute high dose of tranylcypromine, the neurotransmitter is trapped within 5-HT terminals, only a small part of it being available for release (intracellular:extracellular ratio of 5-HT increase in frontal cortex: 11.6 and 5.5 for acute/high-dose and prolonged/low-dose treatments, respectively). In part, increased extracellular availability of 5-HT after long-term MAOI administration is due to desensitization of 5-HT_{1A} autoreceptors and recovery of 5-HT neuron firing frequency. However, an increase in terminal 5-HT release (hippocampus, cortex, and hypothalamus) is seen not only in vivo in the whole animal, but also in vitro in slices containing only 5-HT terminals (Blier and de Montigny, 1985; Blier et al., 1986a,b; Blier and Bouchard, 1994; Mongeau et al., 1994), indicating a 5-HT_{1A}-independent enhancement of neurotransmitter release. Unlike long-term treatment with SSRIs, the sensitivity of 5-HT autoreceptors remains unchanged (Blier et al., 1986a,b; Blier and Bouchard, 1994). The question arising then is, what is the mechanism involved in increasing the releasable amount of 5-HT after long-term MAOI administration? It has long been known that the A form of MAO catalyzes the oxidative deamination not only of 5-HT but also of NE (Hall et al., 1969; Yang and Neff, 1973), and that 5-HT terminals are endowed with inhibitory α_2-adrenergic heteroreceptors (Göthert et al., 1980; Göthert et al., 1981; Maura et al., 1982). More recently, our laboratory has shown that enhanced 5-HT release after sustained MAO-A inhibition correlates with the production of α_2-adrenergic heteroreceptor desensitization by these treatments (Blier et al., 1986a,b; Blier and Bouchard, 1994; Mongeau et al., 1994). Moreover, destruction of the NE system impairs the heteroreceptor desensitization caused by the prolonged administration of the reversible MAO-A inhibitor befloxatone (Mongeau et al., 1994).

If acute administration of MAOIs induces a preferential increase of 5-HT release in midbrain raphe nuclei, then, as in the case of SSRIs, the combined administration of a 5-HT_{1A} antagonist along with a MAOI should produce a greater enhancement of 5-HT availability in the extracellular space of projection areas than the MAOI by itself. Indeed, depressed patients treated with moclobemide or phenelzine and pindolol showed a reduction in the Hamilton Depression Rate Scale score within the first week of combined treatment (Artigas et al., 1994; Blier and Bergeron, 1995). On the other hand, in microdialysis studies in freely moving rats, the acute administration of supramaximal doses of the nonselective inhibitor tranylcypromine (15 mg/kg i.p. which increases motor activity; Celada and Artigas, 1993; Ferrer and Artigas., 1994) have been shown to produce a considerable increase (500–1100%) in extracellular cortical 5-HT, two to four times higher than the long-term administration of 0.5 mg/kg s.c. for 14 days (224% increase). Furthermore, the ratio of DRN:frontal cortex extracellular 5-HT was six after 15 mg/kg and one after 0.5 mg/kg, indicating that the observed 500 to 1100% increase in extracellular cortical 5-HT following the supramaximal dose of tranylcypromine takes place even with a full activation of the negative somatodendritic autoregulatory feedback. Similar results were observed with the acute administration of the nonselective MAOI pargyline (75 mg/kg i.p.) which elicited 10- to 14-fold increases in caudate-putamen and frontal cortex with the same time course as that seen with tranylcypromine (Kalen et al., 1988b; Carboni and Di Chiara, 1989). In the tranylcypromine series of experiments, tissue concentrations of 5-HT were found to reach a plateau at an acute dose of 3 mg/kg, with no further change at 15 mg/kg, whereas the cortical extracellular concentration of neurotransmitter increased 4-fold with the dose increase from 3 to 15 mg/kg (Ferrer and Artigas, 1994). The latter observation indicates that even if MAO-A activity is completely blocked at the low dose of tranylcypromine, it is not the only factor determining extracellular 5-HT availability. The latter interpretation is supported by the fact that the concurrent administration of either brofaromine (10 mg/kg s.c.) or clorgyline (5 mg/kg i.p.) with deprenyl (2.5 mg/kg i.p; Celada et al., 1994; Bel and Artigas, 1995) also increases the extracellular concentration of cortical 5-HT to a much greater extent than the MAO-A inhibitor by itself. This group of observations may be interpreted as an indication that extracellular changes in 5-HT availability are determined not only by MAO-A activity, but also by 5-HT deamination by MAO-B, as well as the capacity of the brain to store 5-HT.

The previous experiments also indicate that it is possible to induce an acute 250 to 400% increase in extracellular 5-HT concentration in projection areas by the concurrent inhibition of MAO-A and MAO-B. If such a strategy were to be applied to achieve a faster therapeutic response or as a potentiation strategy in patients treated with but not responding to MAOIs (i.e., used to induce a sufficient increase in extracellular 5-HT availability in projection areas without inducing dose-related side effects), various facts should be taken into account: 1) MAO-A (contained in catecholaminergic neurons and terminals; Westlund et al., 1985, 1988) is only 1.4 times more concentrated in DRN than in the cortex or hippocampus, whereas the concentration of MAO-B (contained in 5-HT neurons; Levitt et al., 1982; Westlund et al., 1985, 1988) in the midbrain raphe is 3 times higher than in cortico-hippocampal terminal areas (Saura et

al., 1992); 2) MAO-B inhibition has no antidepressant effect by itself (Mann et al., 1984) but contribute to increase terminal 5-HT release when most, if not all, MAO-A activity is blocked; 3) it is possible to produce an almost complete MAO-A blockade without an excessive increase of DRN extracellular concentration of 5-HT (Ferrer and Artigas, 1994); 4) doses of MAO-A/MAO-B inhibitors chosen should favor a terminal versus raphe nuclei blockade of the enzymes; and 5) at doses where nonselective MAOIs produce a significant acute increase in extracellular 5-HT in projection areas, they might also induce collateral side effects. Hence, based on the latter, it could be possible that the minimal dose of selective or nonselective MAO-A inhibitor that completely blocks MAO-A activity (e.g., 3 mg/kg tranylcypromine), administered in combination with one-third of the equivalent dose of 2.5 mg/kg deprenyl used in rats by Artigas' group (to avoid the disproportionate increase in DRN 5-HT), could induce an immediate and significant increase in extracellular 5-HT in projection areas. Under an equivalent treatment, a quicker therapeutic response could be expected but chances of 5-HT syndrome are higher.

One additional question that immediately arises is, how may the "speeding-up strategies" of SSRI/pindolol or MAOI/pindolol manage to overcome inhibitory terminal autoregulation by 5-HT autoreceptors? Given that supramaximal doses of chlomipramine and tranylcypromine produce a 300 to 1100% increase of the basal 2 nM cortical 5-HT concentration (Bel and Artigas, 1992) and that 5-HT concentrations between 10 and 100 nM inhibit 5-HT release from the rat frontal cortex (Middlemiss, 1986; Limberger et al., 1991), an insufficient increase in extracellular concentration of neurotransmitter does not seem an appropriate explanation. The possibility that terminal autoinhibition could in fact be activated but 5-HT still released is not in agreement with the observation that 100 nM 5-HT has been shown to produce 90% inhibition of electrically evoked release of 5-HT in frontal cortex slices (Limberger et al., 1991). Speculating further, it could also be possible that the amount of releasable 5-HT within the terminal vicinity is of such magnitude that even if autoinhibition is activated by the first bursts of arriving action potentials, the amount of initially released 5-HT is already high enough to increase its extracellular availability. Such an explanation would fit only in the case of MAOIs but not of SSRIs (which do not increase intracellular 5-HT availability).

Moreover, another interesting point to be considered is, why, if both prolonged SSRI and MAOI administration increase extracellular availability of 5-HT only following the former, are terminal autoreceptors desensitized? A possible answer to this question could be that by prolonging reuptake time, SSRIs produce a sustained increase in intrasynaptic 5-HT concentration. On the other hand, the increase produced by MAOIs occurs following the arrival of action potentials to the terminal, and hence extracellular 5-HT concentration increases in an "on-off" manner, probably not suitable for producing desensitization.

3. Antidepressants with α_2 Adrenoceptor Antagonistic Properties. Antidepressants with α_2 antagonists like mianserin and (±)-mirtazapine, whose long-term administration increases the duration of suppression of firing of CA_3 pyramidal neurons produced by 5-HT pathway stimulation, have also been shown to desensitize α_2-adrenergic heteroreceptors on 5-HT neurons (Mongeau et al., 1994; Haddjeri et al., 1997). Other studies of the neurochemical effects of long-term mianserin administration (Raiteri et al., 1983a; Schoups and De Potter, 1988) reported no change in the sensitivity of these receptors. A likely explanation for this discrepancy could be that in the latter studies mianserin was injected i.p., whereas in the studies in which they induced α_2-adrenergic heteroreceptor desensitization, antidepressant drugs were delivered continuously via osmotic minipumps implanted s.c.

4. Activation of 5-HT_{1A} Receptors by 5-HT_{1A} Agonists. Direct activation of presynaptic 5-HT_{1A} receptors by 5-HT_{1A} agonists causes inhibition of 5-HT cell firing, synthesis, and release in forebrain areas (Blier and de Montigny, 1987; Hjorth and Magnusson, 1988; Sharp et al., 1989a; Schechter et al., 1990; Godbout et al., 1991). At postsynaptic receptors, biochemical and electrophysiological experiments in the hippocampus (Yocca and Maayani, 1985; Yocca et al., 1986; Andrade and Nicoll, 1987a,b) have shown that clinically available 5-HT_{1A} agonists such as buspirone (Glitz and Pohl, 1991) act as partial agonists and may inhibit cAMP formation pyramidal neuron firing activity. Since upon acute administration there is net decrease in forebrain extracellular availability of 5-HT and their beneficial clinical antidepressant and anxiolytic effects are usually not observed until a few weeks of administration (see Charney et al., 1990; Glitz and Pohl, 1991), it appears that the presynaptic effects of 5-HT_{1A} agonists override their postsynaptic actions. Based on electrophysiological results, it has been proposed that tolerance develops to the autoreceptor-mediated effects (Blier and de Montigny, 1987; Schechter et al., 1990; Godbout et al., 1991), and a combination of normal 5-HT firing activity along with simultaneous activation of postsynaptic normosensitive receptors by the drug and endogenous 5-HT may account for their therapeutic actions (see Haddjeri et al., 1998). Neurochemical studies demonstrating 5-HT_{1A} autoreceptor desensitization after sustained 5-HT_{1A} agonist administration have been less consistent, with positive (Kreiss and Lucki, 1992) and negative results (Sharp et al., 1993a; Söderpalm et al., 1993). A possible explanation for the discrepancy between electrophysiological and neurochemical studies could be that the sensitivity of 5-HT_{1A} autoreceptors was tested directly on 5-HT neurons in the former and systemically in the latter. As

previously discussed, systemic administration of 5-HT_{1A} agonists may modify 5-HT neuron electrophysiological and neurochemical activities acting not only at presynaptic but also postsynaptic 5-HT_{1A} receptors. Furthermore, the hypothesis generated by electrophysiological data predicts that concurrent administration of a 5-HT_{1A} agonist with a selective 5-HT_{1A} autoreceptor blocker should produce rapid enhancement of 5-HT neurotransmission (Blier and de Montigny, 1994), and indeed the administration of buspirone (20 mg/day) with pindolol (2.5 mg/kg thrice daily) produced quick reductions of depressive symptomatology in patients treated with this drug combination (Blier et al., 1997).

E. 5-HT Reuptake

Following release, 5-HT is actively cleared from the synaptic cleft by a high-affinity transporter located on presynaptic neuronal membranes (Kuhar et al., 1972; see Kanner and Schuldiner, 1987; O'Reilly and Reith, 1988), which functions in series with another type of carrier, the vesicular transporter, that sequesters intracellular 5-HT within secretory vesicles. The carriers taking up neurotransmitter from the extracellular space into the neuron are integral membrane proteins with 12 transmembrane spanning domains, they couple reuptake to Na^+ and Cl^- displacement across the plasma membrane, and are encoded by a closely related gene family, the type I or plasma membrane Na^+/Cl^--coupled transporter family, which includes GABA, catecholamine, and 5-HT transporters. The vesicular transporter belongs to a different gene superfamily and will not be considered further (for reviews, see Uhl and Hartig, 1992; Amara and Kuhar, 1993; Lester et al., 1994).

1. Molecular Characteristics of the 5-HT Transporter (SERT). Two main strategies have been used in an attempt to identify the molecular characteristics of the SERT, i.e., biochemical purification and cloning of cDNA coding for the protein. Using digitonin as a detergent, the SERT in rat brain and human platelets has been solubilized and purified in a conformational state that retained a pharmacological profile almost identical with that observed in native membrane preparations (Biessen et al., 1990; Graham et al., 1991; Launay et al., 1992). In a further step, the human placental SERT has been reconstituted after purification, displaying not only the same antidepressant-binding profile as the native carrier but also NaCl-dependent 5-HT transport (Ramamoorthy et al., 1993b). The molecular weight of this functional protein isolated from human placenta is 300,000 (Ramamoorthy et al., 1993b). On the other hand, the other purified proteins have a molecular weight ranging between 55,000 and 78,000 (Biessen et al., 1990; Launay et al., 1992) and that of the cloned SERT itself is about 70,000 (Blakely et al., 1991; Lesch et al., 1993; Ramamoorthy et al., 1993b). Thus, in analogy with the Na^+/glucose transporter (Stevens et al., 1990), it has been proposed that the SERT may exist as an homotetramer (Ramamoorthy et al., 1993b). However, although dimeric concatenated constructs of the transporter have been shown to possess 5-HT transport activity similar to the monomer's, concatenated tetramers have substantially lower activity (Chang et al., 1994). The latter observation does not preclude the existence of functional tetramers since simple functional monomers or dimers could associate and efficiently transport 5-HT.

The recent development of site-specific antibodies has allowed further characterization of the SERT (Lawrence et al., 1995a,b; Qian et al., 1995; Ovalle et al., 1995; Wade et al., 1996). The molecular weight of SERT in immunoprecipitates was found to vary according to the structure of origin: rat platelets, 94,000; rat pulmonary membranes, 80,000; rat brain, 71,000 to 76,000; HeLa cells, 90,000 to 200,000 (Ovalle et al., 1995; Wade et al., 1996). Differential *N*-linked glycosylation has been claimed to account for different molecular weight of CNS and peripheral SERT units. In transfected HeLa cells, the inhibition of glycosylation changes the molecular weight of the transporter, shifting the 90-kDa SERT immunoreactivity band to its presumably unglycosylated state of 56 kDa. In contrast, the mobility of a 200-kDa form of the transporter remained unchanged following glycosylation inhibition. Because unglycosylated monomers have less tendency to aggregate, it has been suggested that the high-weight slow-mobility species may represent a transporter aggregate and that glycosylation may be involved in multimerization (Qian et al., 1995). If this interpretation is correct, the molecular weight of the placental purified transporter (Ramamoorthy et al., 1993b) also suggests the possibility of a tetrameric organization of the transporter (300,000/55,000–78.000 = 5.5–3.8).

Despite the differences in molecular weight, the human CNS and peripheral SERTs are polypeptides encoded by a single gene located on chromosome 17 (Lesch et al., 1993; Ramamoorthy et al., 1993b). The existence of a single hybridizing mRNA, as well as the identity of the cDNAs cloning brain and peripheral rat SERTs, suggest that this is also the case for rodents (Blakely et al., 1991, 1993; Hoffman et al., 1991). Although encoded by a single gene, in humans, unlike rodents, there are different types of SERT mRNAs that have been found in placenta, lung (Ramamoorthy et al., 1993b), and brain (Austin et al., 1994), the abundance of each mRNA species depending on the tissue of origin. The factors determining the expression of different SERT transcripts is presently unknown. In contrast with these results, Lesch et al. (1993) have reported a single hybridizing transcript for human mRNA. These dissenting observations could be explained by differences in probes used.

Analysis of the amino acid sequence of mammalian transporters for 5-HT, NE, and DA shows that 41% of their amino acid residues are identical, homology being highest at the 12 hydrophobic membrane-spanning do-

main levels, lower in the intracytoplasmic carboxyl- and amino-terminals, and particularly low in the large extracellular loop connecting TM3 and TM4, where the 5-HT carrier has two potential *N*-linked glycosylation sites (see Amara and Kuhar, 1993; Rudnick and Clark, 1993). Multiple putative phosphorylation sites by protein kinase C (PKC) and protein kinase A, consistent with rapid postranslational regulation of the SERT, are predominantly found in carboxyl and amino termini (Blakely et al., 1991; Hoffman et al., 1991; Lesch et al., 1993, Corey et al., 1994; Demchyshyn et al., 1994). Moreover, recent reports on the organization of the human *SERT* gene indicate that the latter is endowed with an upstream combination of positive and negative *cis*-acting elements, including the cAMP response element, that may regulate transcription activity via a promoter unit (Lesch et al., 1994; Heils et al., 1995).

2. The Mechanism of 5-HT Uptake. The mechanism of 5-HT uptake has been thoroughly studied in platelets (Rudnick, 1977; Nelson and Rudnick, 1979, 1982), mouse brain plasma membrane vesicles (O'Reilly and Reith, 1988; Reith et al., 1989), human placenta brush-border membrane vesicles (Cool et al., 1990; Ramamoorthy et al., 1993b), and, more recently, after stable expression of cloned SERTs, in different expression systems (Blakely et al., 1991; Hoffman et al., 1991; Ramamoorthy et al., 1993a; Corey et al., 1994; Demchyshyn et al., 1994; Gu et al., 1994; Mager et al., 1994). 5-HT is the specific substrate for the transporter, and K_m values reported across different studies are summarized in Table 8. Reported values for the turnover number (maximal number of 5-HT molecules carried by one transporter in 1 min) in different systems have also been variable: 500 5-HT molecules/porcine SERT in platelet membrane vesicles/min (Talvenheimo et al., 1979), 110 5-HT molecules/rSERT expressed in parental LLC-PK_1 cells/min (Gu et al., 1994), or 30 5-HT molecules/rSERT expressed in *Xenopus* oocytes/min (Mager et al., 1994). Tryptamine and its derivatives, as well as 5-HT derivatives and phenylethyamines such as (+)-amphetamine and PCA, are additional substrates for the SERT (Segonzac et al., 1984; Wölfel and Graefe 1992; Mager et al., 1994). On the other hand, tryptophan, 5-hydroxytryptophan, 5-HIAA, histamine, and the catecholamines NE and DA, at concentrations as high as 10 μM, do not significantly bind to this carrier (Ramamoorthy et al., 1993; Hoffman et al., 1991; Wölfel and Graefe, 1992; Corey et al., 1994; Barker and Blakely, 1995). However, higher concentrations of DA, 20 to 40 μM, have been reported not only to bind to the transporter but to exchange with [^{3}H]5-HT (Ramamoorthy et al., 1993; Wölfel and Graefe, 1992; Corey et al., 1994).

For neurotransmitter influx to occur, all type I plasma membrane transporters, by definition, exhibit absolute requirement for Na^+ in the external medium. The Na^+ concentration gradient has been demonstrated to be the driving force for 5-HT uptake, and it may not be replaced by other cations. If the gradient is experimentally created, independently of Na^+ ATPase activity, 5-HT uptake is insensitive to changes in the latter, indicating that 5-HT and Na^+ fluxes are directly coupled by the transporter (Rudnick, 1977; Kanner and Bendaham, 1985; O'Reilly and Reith, 1988; Ramamoorthy et al., 1993). External Na^+ increases V_{max} and decreases K_m for 5-HT (Cool et al., 1990). In peripheral SERTs, Na^+-5-HT stoichiometry has been considered to be 1:1 (Talvenheimo et al., 1983; Cool et al., 1990). In brain membranes, the increase of 5-HT uptake with Na^+ showed a Hill coefficient of 2, suggesting a requirement of two Na^+ ions for a transport cycle (O'Reilly and Reith, 1988). More recently, Na^+ stoichiometry has been studied in stably expressed rSERT and an hyperbolic function consistent with a 1:1 stoichiometry was found (Gu et al., 1994). If the sodium gradient is kept, external Cl^- increases V_{max} and decreases K_m for 5-HT, fitting a Cl^-:5-HT stoichiometry of 1:1 (Nelson and Rudnick, 1982; Cool et al., 1990; Gu et al., 1994). Intracellular K^+ has also been shown to stimulate 5-HT uptake in platelets, placenta brush-border vesicles, and brain vesicles (Nelson and Rudnick, 1979; Reith et al., 1989; Cool et al., 1990). Only in the case of the neuronal transporter, H^+ could not substitute for K^+ in enhancing uptake (Reith et al., 1989). The overall stoichiometry of a 5-HT uptake cycle, as proposed by Rudnick and Clark (1993), is 5-HT:Na^+:Cl^-:K^+, 1:1:1:1. In their mechanistic model, the authors assume that the transporter may behave like an ion channel with a gate at each face of the membrane, but with only one gate opening at a time. In a first step,

TABLE 8
K_m values for 5-HT uptake reported across different studies

Transporter	K_m	Reference
	nM	
hSERT (platelets)	600	Mager et al., 1994
hSERT (heterologous expression, HeLa cells)	460	Ramamoorthy et al., 1995
hSERT (placental brush-border vesicles)	50–60	Balkovetz et al., 1989
rSERT (heterologous expression, *Xenopus* oocytes)	500–890	Mager et al., 1994
rSERT (heterologous expression, CV-1 cells)	530	Hoffman et al., 1991
rSERT (heterologous expression, HeLa cells)	320	Blakely et al., 1991
rSERT (heterologous expression, basophilic leukemia cells)	220	Kanner and Bendahan, 1985
rSERT (heterologous expression, LLC-PK_1 cells)	150	Gu et al., 1994
rSERT (brain synaptosomes)	60–80	Ross and Renyi, 1975; Angel and Paul, 1984; Wood et al., 1986
mSERT (brain membranes)	60–180	O'Reilly and Reith, 1988

the transporter binds Na^+, Cl^-, and 5-HT on the extracellular face of the membrane. To account for cotransport of the three species, it would undergo conformational change and open the intracellular gate only once all three of them are bound to the permeation channel. K^+ countertransport consists of an inverse conformational change, reorienting the transporter to its "active uptake state" once the K^+ ion is released to the extracellular space. Given that 5-HT is transported in its cationic form (5-HT^+; Keyes and Rudnick, 1982; Rudnick et al., 1989), the 1:1:1:1 stoichiometry should give place to an electroneutral process. In agreement with this concept, in most studies, uptake of 5-HT by mammalian transporters was not affected by membrane potential (see Rudnick and Clark, 1993). However, studies of the rSERT present in basophilic leukemia cells (Kanner and Bendaham, 1985), stably expressed hSERT (Laezza et al., 1994), rSERT (Mager et al., 1994), and *Drosophil*a SERT (Corey et al., 1994) indicate that 5-HT uptake may indeed be electrogenic since it depends on membrane potential and/or generates a transport-associated current. These observations would imply not only that voltage gradients across the membrane may regulate uptake, but also that electrogenic transporters may mediate nonvesicular 5-HT release.

3. Anatomical and Cellular Localization of the SERT. In the periphery, the SERT is expressed in enteric 5-HT neurons (Wade et al., 1996) and non-neuronal cells such as mast cells (Gripenberg, 1976), crypt epithelial cells, and very discretely in enterochromaffin cells (Wade et al., 1996). It is also found in platelets (Rudnick, 1977; Qian et al., 1995), lung membranes (Qian et al., 1995), and maternal brush-border of syncytiotrophoblasts (Cool et al., 1990; Ramamoorthy et al., 1993b).

In the brain, the SERT has been radiolabeled with [^{3}H]imipramine (Langer et al., 1980a,b; Dawson and Wamsley, 1983; Hrdina et al., 1985) and more selective 5-HT uptake inhibitors such as [^{3}H]cyanoimipramine (Wölf et al., 1988; Kovachich et al., 1988; Soucy et al., 1994), [^{3}H]paroxetine (Habert et al., 1985; de Souza and Kuyatt, 1987; Langer et al., 1987; Marcusson et al., 1988), and [^{3}H]citalopram (D'Amato et al., 1987). Although in general the autoradiographic binding pattern of [^{3}H]imipramine was found to be similar to that of [^{3}H]paroxetine (Hrdina et al., 1990) and that of [^{3}H]citalopram (Duncan et al., 1992), there are important regional differences in the density of imipramine- versus SSRI-labeled sites, the former showing a much higher density of binding in forebrain areas such as the cortex and hippocampus. The reason for this discrepancy has been attributed to the fact [^{3}H]imipramine binds to two classes of sites, high and low affinity, but only the high-affinity ones seem related to 5-HT uptake (Moret and Briley, 1986; Marcusson et al., 1986; Hrdina 1987, 1988; see review in D'Amato et al., 1987). For this reason, tritiated SSRIs are the ligands of choice for labeling the brain 5-HT carrier in vitro. Recently, in vitro [^{3}H]cyanoimipramine and [^{3}H]citalopram autoradiograms have been compared to the innervation pattern of 5-HT neurons as marked by [^{3}H]5-HT uptake in rat brain. A similar linear relationship was found for the labeling density of each of these ligands and the density of 5-HT innervation, further indicating the high sensitivity of both [^{3}H]cyanoimipramine and [^{3}H]citalopram to label the SERT (Descarries et al., 1995). [^{3}H]Citalopram binding has also been compared with that of [^{3}H]paroxetine in postmortem human brain tissue, and it was concluded that both of these drugs are highly selective ligands but, because of its higher affinity for the carrier, [^{3}H]paroxetine was suggested as the radioligand of choice for in vitro studies (Arranz and Marcusson, 1994).

Although [^{3}H]paroxetine may be the ligand of choice for in vitro labeling of the SERT, its in vivo distribution of binding resembles that of in vitro imipramine (Biegon and Mathis, 1993), and successful conversion of paroxetine into photon emission tomography (PET) or single photon emission-computerized tomography imaging agents has not been accomplished despite its very high potency for uptake inhibition (see Scheffel et al., 1992). Other drugs with high affinity for the uptake site, such as cyanoimipramine, sertraline, citalopram, or fluoxetine, have been labeled with ^{11}C but also displayed relatively low specific to nonspecific binding ratios in vivo (Hashimoto et al., 1987; Lasne et al., 1989; Scheffel and Ricaurte, 1990; Hume et al., 1991). McN-5652-Z (*trans*-1,2,4,5,6,10β-hexahydro-6-[4-(methylthio)phenyl]pyrrolo[2,1-a]isoquinoline) is another potent blocker of 5-HT uptake that has been ^{11}C-tagged and assessed as a PET radiotracer in mouse brain (Shank et al., 1988). This study suggested that McN-5652-Z may label 5-HT uptake sites in vivo with high target:nontarget ratio, holding as a promising radiotracer for human PET studies (Suehiro et al., 1993). In vivo imaging of the 5-HT carrier has also been assessed in rat and nonhuman primate brain with the cocaine analog [(^{123}I)β-CIT ([^{123}I]methyl-3β-(-4-iodophenyl)tropane-2β-carboxylate; Scheffel et al., 1992; Laruelle et al., 1993). Although this cocaine analog binds to the DA transporter as well as the SERT, both sites may be discriminated because of kinetic differences in the way the ligand is taken up or washed out from rich 5-HT and DA innervation areas (Laruelle et al., 1993). Indeed, in a recent PET study in healthy human volunteers, the SERT was distinctly recognized in the medial frontal cortex, brainstem, hypothalamic area, and visual occipital cortex 1 h after injection, and DA transporters were recognized in the basal ganglia 20 h later (Kuikka et al., 1995). In this same study, 5-HT uptake sites were found to be reduced in the frontal cortex of a patient with depression and increased in the occipital cortex of a patient with panic disorder.

Cellular localization of SERTs in the CNS has been accomplished by using site-specific antibodies (Lawrence et al., 1995a,b; Ovalle et al., 1995; Qian et al., 1995). Immunocytochemistry using antibodies directed

against sites on the second and third extracellular loops of the 5-HT carrier revealed both neuronal and glial staining in areas of the rat brain containing 5-HT somata and terminals (dorsal raphe and hippocampus) (Lawrence et al., 1995b). In contrast, Qian et al. (1995), using an antibody developed against the intracellular N terminus, found no evidence of glial staining. In this case, SERT-immunoreactive somata and a dense network of SERT-immunoreactive processes were observed in the DRN and on 5-HT terminals in the CA_{2-3} region of the hippocampus (Qian et al., 1995). It may then be possible that either the glial SERT expression in adult rat brain is not very abundant or the epitope on the N terminus is not expressed (or masked) in the glial SERT. Thus, SERT expression in adult brain astrocytes remains a matter of debate. Using colocalization techniques for glial fibrillary acidic protein and radioactivity for [^{3}H]5-HT, 5-HT uptake activity has been found in primary astrocyte culture (Katz and Kimelberg, 1985; Kimelberg and Katz, 1985) and in 50% (frontal cortex) to 80% (periventricular region) of adult rat brain astrocytes (Anderson et al., 1992). Furthermore, Artigas et al. (1995) found that, after intracortical perfusion of 5-HT, 5,7-dihydroxytryptamine (5,7-DHT)-pretreated rats displayed in vivo SSRI-sensitive 5-HT uptake similar to that of control rats. Our group, on the other hand, using an electrophysiological and in vitro uptake paradigm, found no effect of paroxetine in 5,7-DHT-treated rats (Piñeyro et al., 1994). Our results are in agreement with in situ hybridization studies that have failed to detect any hybridization signal for mRNA in glial cells (Fujita et al., 1993). Like SERT immunoreactivity, SERT mRNA is present in neurons of caudal linear nucleus, DRN, MRN, and caudal 5-HT nuclei, matching the distribution of cell bodies but not that of terminals (Fujita et al., 1993; Austin et al., 1994). Only in one study, in which reverse transcription-polymerase chain reaction was used for amplification, a detectable level of SERT mRNA expression was found in the frontal cortex, hippocampus, and neostriatum, apart from the abundant expression observed in the midbrain raphe complex (Lesch et al., 1993). Such an observation indicates that SERT mRNA may be specifically targeted to different projection areas where it can initiate transporter synthesis, and this opens for consideration the possibility that mRNA transcription could be locally regulated.

4. Pharmacological Properties of the SERT. The SERT is the pharmacological target for various therapeutic and abused substances. Compounds that block 5-HT reuptake such as tricyclic antidepressants and SSRIs are in the first group, whereas stimulants such as amphetamine and its derivatives, which block 5-HT uptake and promote release, are part of the latter category. Cocaine, although it binds and blocks the 5-HT carrier, is believed to exert most of its behavioral effects by blocking DA rather than 5-HT uptake (Woolverton and Kleven, 1992; Barker and Blakely, 1995; Caron, 1996).

5-HT and NE transporters share their sensitivity for tricyclic antidepressants, tertiary amines such as imipramine and chlomipramine being more potent at the SERT, and secondary amine tricyclics (e.g., desipramine, nortriptyline) at the NE transporter (see Hyttel, 1982; Thomas et al., 1987; Langer and Schoemaker, 1988; Bolden-Watson and Richelson, 1993). [^{3}H]Imipramine-binding sites were among the first antidepressant-binding sites to be described both on 5-HT neurons and platelets (Raisman et al., 1979; Langer et al., 1980a,b). Based on early observations in which the binding of this radioligand was inhibited in a complex manner by 5-HT and SSRIs but competitively by imipramine itself (Langer and Raisman, 1983; Sette et al., 1983), it was initially proposed that imipramine-like antidepressants (imipramine, amitriptyline, or chlomipramine) would act by allosterically regulating the function of the transporter without directly binding to the substrate recognition site (Langer and Raisman, 1983). Conversely, the observation that imipramine and other tricyclics as well as SSRIs inhibit the binding of the selective SERT ligand [^{3}H]paroxetine in a competitive manner (Habert et al., 1985; Marcusson and Eriksson, 1988; Graham et al., 1989; Marcusson et al., 1989, 1992) later suggested that there is a single or at least overlapping binding site for tricyclic and nontricyclic 5-HT uptake inhibitors on the SERT. The idea of an overlapping binding site for the substrate, tricyclic and nontricyclic uptake inhibitors is further supported by the following observations: 1) in membranes from rat and human brain, 5-HT produces competitive displacement not only of [^{3}H]imipramine but also of [^{3}H]paroxetine which in turn may be displaced, fitting a single-site binding model by citalopram, norzimelidine, paroxetine, fluoxetine, indalpine, chlomipramine, and desipramine (Habert et al., 1985; Marcusson et al., 1988, 1989, 1992); 2) [^{3}H]5-HT uptake by dSERT is inhibited in a monophasic manner by paroxetine > fluoxetine > citalopram > cocaine[3] 5-HT > desipramine > imipramine (Demchyshyn et al., 1994); 3) Na^+ ions are needed for paroxetine and imipramine binding, as well as for 5-HT binding and translocation (Wood et al., 1986; Mann and Hrdina, 1992); and 4) preincubation with imipramine protects against the reduction in total [^{3}H]paroxetine binding caused by the sulfydryl group alkylating agent NEM (Graham et al., 1989). It is possible then that the early observations by Langer's group could be explained by taking into account the existence of two components for [^{3}H]imipramine-specific binding, i.e., high-affinity, Na^+-dependent binding and low-affinity, Na^+-independent binding, as defined using desipramine to determine nonspecific binding (Hrdina, 1984, 1987, 1988). Indeed, the high-affinity, Na^+-dependent component of [^{3}H]imipramine binding is completely displaced by 5-HT and nontricyclic reuptake blockers in brain and platelets (Marcusson et al., 1986; Hrdina, 1988; Humphreys et al., 1988).

Even if the above-mentioned findings support the existence of a common recognition site for 5-HT, tricyclics, and nontricyclic antidepressants, there is also compelling evidence indicating that, although overlapping, these sites are distinct, sharing some but not all interacting chemical groups: 1) chemical modification of the platelet SERT with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, a reagent that links carbonyl moieties to vicinal amino groups, significantly reduces the total number of [^{3}H]imipramine-binding sites, an effect that is prevented by preincubation with imipramine and 5-HT but not fluoxetine and citalopram (Biessen et al., 1988); 2) oxidation of the SERT in human platelets by the thiol reagent phenylarsine oxide reduces [^{3}H]imipramine binding by 90%, an effect that is prevented by the preincubation with the tricyclic drugs imipramine, cyanoimipramine, chlomipramine, and amitriptyline but not by the nontricyclic reuptake blockers citalopram, fluoxetine, femoxetine, and zimelidine (Biessen et al., 1988). On the other hand, in rat cortical membranes, preincubation with imipramine did protect [^{3}H]paroxetine-binding sites from NEM inactivation (Graham et al., 1989); 3) the sulfydryl-reducing agent dithiothreitol increases the affinity of the human platelet SERT for [^{3}H]imipramine but not for [^{3}H]paroxetine (Tarrant and Williams, 1995); 4) antisera directed against the second extracellular loop of the SERT produce a dose-dependent inhibition of [^{3}H]5-HT uptake but have no effect on [^{3}H]citalopram binding (Lawrence et al., 1995b); and 5) incubation of [^{3}H]citalopram, [^{3}H]imipramine, or [^{3}H]paroxetine in the presence of high micromolar concentrations of 5-HT, citalopram, or paroxetine may induce very different types of changes in the dissociation kinetics of each radioligand, e.g., 200 μM citalopram attenuated the dissociation of [^{3}H]citalopram four times more than that of [^{3}H]paroxetine, whereas paroxetine has an opposite effect, increasing the dissociation rate of [^{3}H]imipramine (Wennogle and Meyerson, 1985; Humphreys et al., 1988; Plenge et al., 1990, 1991; Plenge and Mellerup, 1991). This last set of observations is consistent with the existence of low-affinity sites that may modulate the binding status of the high-affinity site. Further support for allosterism is given by the fact that paroxetine (although in the low nanomolar range) may decrease the affinity of the SERT for [^{3}H]cocaine (Akunne et al., 1992). Cocaine, in turn, binds to a site that may be distinguished from the substrate/antidepressant site because binding of the latter but not that of 5-HT or antidepressants is insensible to Cl^- and inhibited by H^+ (Wall et al., 1993). Conversely, the ability of 5-HT to competitively displace cocaine analogs such as 2-β-[^{3}H]carbomethoxy-3-β-[4-fluorophenyl]tropane and β-[^{125}I]citalopram argues that cocaine and substrate sites, if not the same, are closely related (Rudnick and Wall, 1991; Wall et al., 1993). Other drugs of abuse such as neurotoxic amphetamine derivatives (MDMA, MDA, PCA) also induce competitive displacement of [^{3}H]imipramine and inhibition of [^{3}H]5-HT transport (Rudnick and Wall, 1992a). Site-directed mutagenesis and chimera construction should help determine which amino acid residues on the transporter interact with all or most of the drugs and which are unique to each ligand. Such studies have suggested that 1) phenylalanine 586 located on the 12th transmembrane domain could be responsible for high-affinity recognition of imipramine (present in human but not *Drosophila* SERT); 2) (+)-amphetamine interacts with multiple residues in this same transmembrane domain; and 3) contact sites for citalopram have been localized to the second transmembrane domain (Barker et al., 1995).

5. Tianeptine, a Class by Itself? Tianeptine is a tricyclic agent (dibenzothiazepine nucleus) with a long (aminoheptanoic acid) lateral chain (Labrid et al., 1988). In France, it is indicated for the treatment of "neurotic and reactional depressive conditions" and has been claimed to be a unique type of antidepressant that produces its effect by enhancing 5-HT uptake.

In rat cortical and hippocampal synaptosomes (Mennini et al., 1987; Fattaccini et al., 1990), as well as in rat and human platelets (Kato and Weitsch, 1988; Chambda et al., 1991), the increase in 5-HT uptake induced by tianeptine is secondary to a 20 to 30% increase in V_{max}. Tianeptine does not displace [^{3}H]paroxetine, [^{3}H]imipramine, nor [^{3}H]*d*-fenfluramine and does not produce in vitro effects on 5-HT uptake (Kato and Weitsch, 1988). The increase in V_{max} observed ex vivo at least 1 h after acute administration of tianeptine (Mennini et al., 1987) should then be an indirect effect. This interpretation is supported by the observation that, if given 1 h before sacrifice at a dose similar to the one used in ex vivo experiments (10 mg/kg i.p.) in which V_{max} is increased, tianeptine does not modify [^{3}H]imipramine binding to rat cortical membranes (Romero et al., 1992). Also, when given acutely (10 mg/kg) or chronically (10 or 20 mg/kg/day for 14 days), tianeptine has no significant effect on 5-HT uptake in mesencephalic synaptosomes where SERTs are most abundant (Mennini et al., 1987). In keeping with this observation, neither acute nor sustained tianeptine administration modify 5-HT neuron firing frequency (Dresse and Scuvée-Moreau, 1988; Piñeyro et al., 1995c). Furthermore, electrophysiological data from our laboratory also indicate that the increase in the firing activity of hippocampal pyramidal neurons after acute administration of tianeptine does not depend on the integrity of 5-HT terminals nor the presence of SERTs, since the effect of the drug is not modified by 5,7-DHT lesions (Piñeyro et al., 1995c). Moreover, analysis of the effect of sustained tianeptine administration also indicates that its effect on 5-HT uptake is not always reproducible. Sustained tianeptine administration increases 5-HT uptake in rat platelets and brain synaptosomes (10–20 mg/kg/day for 14 days; Mennini et al., 1987; Kato and Weitsch, 1988) but not in rat brain slices (20 mg/kg/day for 14 days; Piñeyro et al., 1995d) or human

platelets (37.5 mg/kg/day for 10 or 28 days; Chambda et al., 1991). In the brain, the 5-HT uptake-enhancing effect of tianeptine occurs after a 72-h but not a 24-h washout (Mennini et al., 1987; Mocaër et al., 1988). In platelets, on the other hand, a 24-h washout allows the demonstration of a 30% increase in V_{max} (Kato and Weitsch, 1988). Results from certain binding studies indicate that doses of 10 to 20 mg/kg/day for 14 days (which may enhance cortical and hippocampal 5-HT uptake) produce no change in hippocampal [^{3}H]imipramine- or [^{3}H]paroxetine-binding parameters (Mennini et al., 1987; Mennini and Garattini, 1991; Frankfurt et al., 1993), whereas other studies have shown not only a decrease in B_{max} for [^{3}H]paroxetine-binding sites in the cortex, hippocampus, and DRN (Watanabe et al., 1993; Kuroda et al., 1994) but also a decrease in SERT mRNA in DRN (Kuroda et al., 1994). Prenatal exposure to tianeptine (20 mg/kg/day for 14 days) has also been shown to reduce B_{max} for [^{3}H]imipramine in rat cortex (Romero et al., 1992).

Except for the above-mentioned biochemical studies that have assessed the actual effect of tianeptine on 5-HT uptake, evidence supporting the idea that this drug enhances 5-HT reuptake is indirect. One hour after its acute administration (10 mg/kg i.p.), tianeptine was shown to increase brain tissue concentration of 5-HIAA without modifying tissue levels of 5-HT (Fattaccini et al., 1990). At a similar dose, it has also been shown to induce an increase in extracellular 5-HIAA in the hippocampus, hypothalamus, and medullary dorsal horn (de Simoni et al., 1992; Puig et al., 1993). Interestingly, a dose of 20 mg/kg (i.p.) had the opposite effect on extracellular 5-HIAA in the rat hippocampus (Mennini and Garattini, 1991) but increased plasma 5-HIAA levels (Ortiz et al., 1991). The observed increases in 5-HIAA have been interpreted as an increase in intracellular 5-HT turnover secondary to enhanced 5-HT uptake. The opposite effects of 10-mg/kg and 20-mg/kg doses in the hippocampus remain unexplained. Another common approach that has been used to unveil the site of action of tianeptine is its interaction with drugs that are known to modify 5-HT reuptake activity (Fattaccini et al., 1990; Ortiz et al., 1991; de Simoni et al., 1992; Datla and Curzon, 1993). In keeping with its 5-HT uptake-enhancing capacity, tianeptine, given 30 min after different SSRIs or in combination with 5-hydroxytryptophan (5-HTP), respectively reduced or prevented the increase in plasma or extracellular brain 5-HT caused by the aforementioned treatments. In both cases, consistent with the interpretation that tianeptine increases 5-HT uptake and intracellular deamination, its administration potentiated the increase in 5-HIAA, respectively, induced by SSRIs in plasma or 5-HTP in the extracellular cortical fluid (Ortiz et al., 1991; Datla and Curzon, 1993). On the other hand, opposite results have also been observed. In the rat hippocampus, as in many other brain regions, SSRI administration reduced extracellular availability of 5-HIAA, and tianeptine had no effect on this reduction (de Simoni et al., 1992). Furthermore, in the same study, a metabolite of the 5-HT-releasing drug fenfluramine produced the same increase in 5-HIAA independent of whether rats had been pretreated with tianeptine or not (de Simoni et al., 1992). Acute tianeptine administration was also without effect on the 5-HT depletion caused by *d*-fenfluramine in the cortex and striatum (Fattaccini et al., 1990) and on the increase caused by paroxetine in the time it takes CA_3 pyramidal neurons to recover their firing activity following microiontophoretic application of 5-HT (Piñeyro et al., 1995c). Lack of interaction between tianeptine and drugs that modify SERT activity should be also confronted with yet other findings suggesting that such an interaction may exist: 1) tianeptine no longer induced an increase in tissue 5-HIAA when fenfluramine was previously administered (Fattaccini et al., 1990), 2) in the rat hippocampus the administration of sertraline after tianeptine reverted the enhancing effect of the latter on 5-HIAA production (de Simoni et al., 1992), and 3) after its sustained administration, tianeptine antagonizes the increase in the time of recovery of firing of CA_3 pyramidal from microiontophoretic applications of 5-HT produced by the SSRI paroxetine (Piñeyro et al., 1995d). The effect of tianeptine on basal 5-HT release has also been assessed, and these experiments indicate that neither its acute nor its sustained administration has an effect on this parameter (Mennini et al., 1987; Whitton et al., 1991b). However, both acute and sustained administration reduce K^+-induced 5-HT release from the rat brain in vitro (IC_{50} 2 μM in cortex and 0.4 μM in hypothalamus) and in vivo (Mocaër et al., 1988; Whitton et al., 1991b). Most interestingly, as reported by Mocaër et al. (1988), the in vitro effect of tianeptine is partially blocked by methiothepin (1 μM), once again suggesting that a straight-forward interpretation of a decreased 5-HT output due to increased 5-HT uptake may not be the only explanation possible for this effect. Also supporting this view, Bolaños-Jiménez et al. (1993) have recently reported that tianeptine dose-dependently reduced the effect of the 5-HT_{1B} agonist CGS 12066B on 5-HT release. This group has also shown that without modifying basal outflow, tianeptine (100 μM) may inhibit [^{3}H]ACh release from hippocampal rat synaptosomes (Bolaños-Jiménez et al., 1993). Interestingly, in keeping with the high concentrations used in this study, doses of 30 mg/kg i.p. but not lower ones were found to inhibit in vivo ACh release from the rat hippocampus (Bertorelli et al., 1992).

From the previous analysis, it can be concluded that there is no simple explanation for the effects of tianeptine on the 5-HT system. One of the most consistent observations is that acute as well as prolonged administration of tianeptine may reduce 5-HT neurotransmission when the latter is enhanced by 5-HTP, K^+-evoked release, or SSRIs. Moreover, in the study by Mennini et al. (1987), in which cortical and hippocampal synapto-

somes were shown for the first time to increase V_{max}, the lowest 5-HT concentration used was 40 nM (at least 15 times higher than the normal extracellular brain concentration of 5-HT), and in that by Fattaccini et al. (1990), the [^{3}H]5-HT concentration was at least 5 times higher than the basal extracellular 5-HT. These neurochemical observations are supported by behavioral studies in which tianeptine has been shown to reduce some of the symptoms of 5-HTP-induced 5-HT syndrome (de Simoni et al., 1992; Datla and Curzon, 1993). Moreover, tianeptine has been shown to attenuate behaviors that have been attributed to stress-induced increases in 5-HT activity, i.e., it attenuates stress-induced open-field behavioral deficits without altering basal locomotion parameters (Broqua et al., 1992; Whitton et al., 1991a; Fontanges et al., 1993). It also abolishes stress-induced decreases in hypothalamic corticotrophin-releasing factor (Delbende et al., 1994), it suppresses decreases in glucocorticoid type I hippocampal receptors induced by isolation rearing without affecting basal levels of the latter nor type II receptors (McEwen, 1991, 1992), and it prevents stress- and corticosterone-induced reduction in CA_3 pyramidal neuron apical tree (Watanabe et al., 1992, 1993). Tianeptine has not been found to alter basal ACTH or corticosteroid plasma levels (Delbende et al., 1993, 1994; Watanabe et al., 1993), nor to modify the efficacy of 5-HT neurotransmission in basal conditions (Piñeyro et al., 1995c). On the other hand, it reduces the recovery time necessary for CA_3 pyramidal neurons to recover their firing activity following microiontophoretic applications of high currents of concentrated 5-HT (Piñeyro et al., 1995c).

The next question that naturally arises is whether the "protective" effect of tianeptine against stress-induced changes is linked to its uptake-enhancing capacity. A recent study by Mennini et al. (1993) directly addressed this question, indicating that tianeptine (10 mg/kg i.p. 1 h before stress) antagonizes the decrease in 5-HT uptake caused by acute noise stress. However, tianeptine has also been shown to induce specific changes in other brain monoamines involved in the response to stress: 1) acute tianeptine administration decreases the firing activity of NA neurons in locus ceruleus without altering the activity of DRN 5-HT neurons (Dresse and Scuvée-Moreau, 1988); 2) without producing marked changes in the 5-HT system, short-term administration of tianeptine (10 mg/kg/day for 4 days) increases NE content and decreases NE turnover in specific nuclei related with mood, i.e., preoptic area, DRN, and sensory cortex (Frankfurt et al., 1994); 3) its sustained administration, like that of desipramine, antagonizes the stress-induced increase in tyrosine hydroxylase mRNA in the NA nuclei (see McEwen, 1991); and 4) acute and prolonged tianeptine treatment may increase extracellular DA concentrations in striatum and nucleus accumbens in a 5-HT-independent manner (Invernizzi et al., 1992), as well as increase DA turnover in the prefrontal cortex (Louilot et al., 1990). Hence, these results further indicate that an interaction with the 5-HT system may not be the only mechanism by which tianeptine attains its stress-protecting effects.

Finally, clinical proof of the antidepressant efficacy of tianeptine has been recently reviewed by Wilde and Benfield (1995). It was concluded that, according to the available information, the antidepressant efficacy of this new drug, administered in the short term, appears similar to that of amitriptyline, imipramine, and fluoxetine, and in patients with coexisting anxiety and depression, tianeptine could be superior to maprotiline. However, it should be noticed that 1) most of the studies lacked a placebo-controlled group; 2) in amitriptyline and maproptyline studies, as well as in some of those in which tianeptine was compared with imipramine, optimal dosages of the standard drugs were not used; 3) in multicenter double-blind studies, in which the efficacy of long-term tianeptine treatment was assessed, efficacy was evaluated taking into account only those patients who completed the treatment, even if 65% of dropouts under tianeptine treatment abandoned the study due to ineffective treatment; and 4) comparative trials of tianeptine and SSRIs other than fluoxetine are needed to further define its role in the treatment of depression. Based on basic and clinical facts, it appears that it may be premature to claim that "tianeptine is a new antidepressant class with a unique 5-HT uptake enhancing profile". Furthermore, if indeed 5-HT uptake is stimulated by the drug in conditions in which 5-HT neurotransmission is enhanced, then it would seem unlikely that this be the mechanism involved in its antidepressant action since depression is a condition in which the 5-HT system is frequently deficient (see Maes and Meltzer, 1995). It may, however, protect against the deleterious effects that different stressful conditions may impose on the 5-HT system of a depressed patient.

F. Regulation of 5-HT Uptake Activity by Antidepressant Drugs

The results from radioligand and functional studies following prolonged antidepressant treatments have often been found to be controversial (Table 9), and the reasons for this controversy have been attributed to three main limitations: 1) the use of [^{3}H]imipramine as a radioligand which, due to its binding to heterogeneous sites, may confound interpretation of results (it is worth noting that when the effect of long-term antidepressant administration was assessed on high- and low-affinity [^{3}H]imipramine sites, treatment-induced decreases in affinity for imipramine were observed at both sites; Hrdina, 1987); 2) examination of the effects of long-term antidepressants being limited in most cases to cortical or hippocampus homogenates which, unlike autoradiographic analysis, prevents modest changes in discrete brain areas to be assessed; and 3) the use of low doses and mainly administration routes (i.p. and s.c. injec-

TABLE 9
Effect of different antidepressant treatments on functional and binding properties of the 5-HT transporter

Treatment	Dose/Route	Ligand	Effect Observed	Reference
ECS	Once	[^{3}H]paroxetine	Increase in number of binding sites in cortical homogenates, not hippocampus	Hayakawa et al., 1995
	1/d × 14 ds	[^{3}H]paroxetine	Increase in number of binding sites in cortical homogenates, not hippocampus	Hayakawa et al., 1995
	1 every 2 ds × 10 ds	[^{3}H]paroxetine	No change in frontal cortex homogenates	Cheetham et al., 1993
	1/d × 10 ds	[^{3}H]paroxetine	No change in cortex homogenates	Gleiter and Nutt, 1988
	1/d × 10 ds	[^{3}H]imipramine/functional	No change in V_{max} or B_{max} in cortical synaptosomes or homogenates	Craig et al., 1986
	1/d × 7 ds	[^{3}H]imipramine	Increase in B_{max} in cortical homogenates	Barkai, 1986
Imipramine	20 mg/kg/d × 14 ds (i.p.)	[^{3}H]paroxetine	No change in hippocampal or cortical homogenates	Hayakawa et al., 1995
	5 mg/kg/d × 21 ds (i.p.)	[^{3}H]paroxetine	No change in frontal cortex homogenates	Dewar et al., 1993
	20 mg/kg/d × 21 ds (i.p.)	[^{3}H]imipramine	Increase in V_{max} and decrease in B_{max} in synaptosomes and homogenates	Barbaccia et al., 1983
	3 mg/rat/d × 5 wk (p.o.)	[^{3}H]imipramine	No change in cortical homogenates	Plenge and Mellerup, 1988
	20 mg/kg/d × 21 ds (i.p.)	[^{3}H]imipramine	No change in V_{max} or B_{max} in cortical synaptosomes or homogenates	Marcusson et al., 1988
	20 mg/kg/d × 10 ds (i.p.)	[^{3}H]imipramine	Decrease in B_{max} in hippocampal homogenates No change in cortical homogenates	Kinnier et al., 1980
	5 mg/kg/d × 21 ds (s.c. minipumps)	mRNA hybridization	Decrease in midbrain raphe complex	Lesch et al., 1993a
	20 mg/kg/d × 16 or 32 ds (i.p.)	mRNA hybridization	No change in midbrain homogenates	Spurlock et al., 1994
	10 mg/kg/d × 21 ds (i.p.)	mRNA hybridization	Increase in optical density in DRN autoradiograph	Lopéz et al., 1994
Desipramine	10 mg/kg/d × 14 ds (p.o.)	[^{3}H]paroxetine	No change in frontal cortex homogenates	Cheetham et al., 1993
	20 mg/kg/d × 21 ds (i.p.)	[^{3}H]imipramine	Decrease in B_{max} incortical homogenates	Barbaccia et al., 1983
	10 mg/kg/d × 10 ds (i.p.)	[^{3}H]imipramine	No change in midbrain raphe area or limbic terminal projection areas	Biegon, 1986
	13.6 mg/kg/d × 22 ds (p.o., in drinking water)	[^{3}H]imipramine	Decrease in B_{max} in cortical homogenates	Butler, 1987
	5 mg/kg/d × 21 ds (s.c., via osmotic minipump)	[^{3}H]imipramine	No change in midbrain raphe complex	Raisman et al., 1980
	10 mg/kg/d × 21 ds (i.p.)	Functional	No change in cortical or amygdala synaptosomes	Lesch et al., 1993
Trimipramine	5 mg/kg/d × 21 ds (i.p.)	[^{3}H]paroxetine	No change in frontal cortex homogenates	Dewar et al., 1993
Protriptyline	20 mg/kg/d × 21 ds (i.p.)	[^{3}H]cyanoimipramine	No change in cortex or hippocampus autoradiograms	Kovachich et al., 1992
Amitriptyline	10 mg/kg/d × 14 ds (p.o.)	[^{3}H]paroxetine	No change in frontal cortex homogenates	Cheetham et al., 1993
Nortryptiline	20 mg/kg/d × 21 ds (gastric intubation)	[^{3}H]imipramine/functional	No change in V_{max}, decrease in affinity for imipramine in frontal cortex homogenates	Hrdina, 1987b
Chlomipramine	20 mg/kg/d × 19 ds (i.p.)	[^{3}H]paroxetine	No change in K_D or B_{max} in cortex and hippocampus homogenates	Graham et al., 1987
	5 mg/kg/d × 19 ds (s.c. via osmotic minipump)	[^{3}H]paroxetine	No change in K_D or B_{max} in cortex and hippocampus homogenates	
	5 mg/kg/d × 21 ds (s.c. via osmotic minipump)	mRNA hybridization	No significant change in midbrain homogenates	Lesch et al., 1993
Clorgyline	1 mg/kg/d × 18 ds (i.p.)	[^{3}H]paroxetine	No change in K_D or B_{max} in cortical and hippocampus homogenates	Graham et al., 1987
	1 mg/kg/d × 21 ds (s.c. via osmotic minipump)	mRNA hybridization	No change in midbrain raphe complex	Lesch et al., 1993
	4 mg/kg/d × 21 ds (i.p.)	mRNA hybridization	Increase in optical density in DRN autoradiographs	Lopéz et al., 1994
Pargyline	100 μM/kg/d × 21 ds (i.p.)	[^{3}H]imipramine	No change in K_D and B_{max} in cortical homogenates	Zsilla et al., 1983
Phenelzine	5 mg/kg/d × 21 ds (i.p.)	[^{3}H]cyanoimipramine	Increase in binding in perirhinal cortex autoradiographs	Kovachich et al., 1992
Tranylcypromine	10 mg/kg/d × 14 ds (p.o.)	[^{3}H]paroxetine	No change in frontal cortex homogenates	Cheetham et al., 1993

TABLE 9
Continued

Treatment	Dose/Route	Ligand	Effect Observed	Reference
Deprenyl	0.5 mg/kg/d × 18 ds (i.p.)	[^{3}H]paroxetine	No change in K_D or B_{max} in cortical and hippocampal homogenates	Graham et al., 1987
	1 μM/kg/d × 21 ds (s.c. injected daily)	[^{3}H]imipramine	Increase in B_{max} in cortical and hippocampal homogenates	Zsilla et al., 1983
	0.25 mg/kg/d × 21 ds (i.p.)	[^{3}H]cyanoimipramine	Increase in binding in dentate gyrus and amygdala autoradiograph	Kovachich et al., 1992
Citalopram	10 mg/kg/d × 14 ds (p.o.)	[^{3}H]paroxetine	No change in frontal cortex homogenates	Cheetham et al., 1993
	20 mg/kg/d × 21 ds (i.p.)	[^{3}H]cyanoimipramine	No change in autoradiographs	Kovachich et al., 1992
	20 mg/kg/d × 19 ds (i.p.)	[^{3}H]cyanoimipramine	No change in frontal cortex homogenates	Graham et al., 1987
	10 mg/kg/d × 16 or 32 ds (i.p.)	mRNA hybridization	No change in mesencephalic homogenates	Spurlock et al., 1994
	40 mg/kg/d 13 ds (p.o., given with diet)	Functional	No change in whole-rat brain synaptosomes	Hyttel et al., 1984
Fluoxetine	2 mg/kg/d × 21 ds (i.p.)	[^{3}H]paroxetine	No change in frontal cortex synaptosomes	Dewar et al., 1993
	10 mg/kg/d × 21 ds (i.p.)	[^{3}H]paroxetine	Increase in binding in cortical and hippocampal autoradiographs	Hrdina and Vu, 1993
	10 mg/kg/d × 21 ds (i.p.)	[^{3}H]imipramine/functional	Decrease in V_{max} and affinity binding in frontal cortex synaptosomes and homogenates	Hrdina, 1987b
	2.5 mg/kg/d × 21 ds (s.c. via osmotic minipump)	mRNA hybridization	Decrease in midbrain raphe complex homogenates	Lesch et al., 1993a
Fluovoxamine	50 mg/kg/d × 16 or 32 ds (i.p.)	mRNA hybridization	No change in midbrain homogenates	Spurlock et al., 1994
Paroxetine	10 mg/kg/d × 21 ds (s.c., via osmotic minipump)	[^{3}H]paroxetine/functional	Decrease in B_{max} in cortical and hippocampal homogenated. Decrease in 5-HT uptake in hippocampal slices	Piñeyro et al., 1994
Sertraline	10 mg/kg/d × 21 ds (i.p.)	[^{3}H]cyanoimipramine	Decrease in binding in amygdala and perirhinal cortex autoradiograph	Kovachich et al., 1992
	10 mg/kg/d × 21 ds (i.p.)	Functional	Decrease in V_{max} in cortex and amygdala synaptosomes	Butler, 1987
Zimelidine	10 mg/kg/d × 14 ds (p.o.)	[^{3}H]paroxetine	No change in frontal cortex homogenates	Cheetham et al., 1993
	20 mg/kg/d × 21 d (i.p.)	[^{3}H]imipramine	No change in V_{max} or B_{max} in cortical synaptosomes and homogenates	Marcusson et al., 1988

d, day; ds, days.

tions; p.o. administration) that cause important fluctuations (peak and trough) in the plasmatic concentration of antidepressant drugs may also account for a high proportion of negative results. Nevertheless, if these limitations are taken into account, certain general conclusions may be drawn.

When selective ligands are used to label the SERT, repeated electroconvulsive shocks (ECS) and long-term MAOI administration were the only types of treatment that consistently showed an increase in the number of SERTs (Kovachich et al., 1992; Hayakawa et al., 1995). The use of [^{3}H]imipramine as a radioligand has shown increases in SERT sites after ECS or deprenyl administration (Zsilla et al., 1983; Barkai, 1986). The studies that yielded negative results were either performed in homogenates, used smaller number of ECS, or p.o./i.p. treatments (Zsilla et al., 1983; Graham et al., 1987; Gleiter and Nutt, 1988; Cheetham et al., 1993). In hybridization as in binding studies either no change or increased mRNA hybridization was found in the midbrain raphe complex after prolonged clorgyline administration (Lesch et al., 1993; Lopéz et al., 1994). Interestingly, in studies yielding positive results, treatment was given i.p. (López et al., 1994), whereas in those in which no change was observed, steady drug plasma levels were achieved by using osmotic minipumps (Lesch et al., 1993). The fact that the dose used in the latter (Lesch et al., 1993) was four times smaller than the one used in the former (López et al., 1994) may explain the results obtained. In the case of SSRIs, functional studies show a decrease in V_{max} in cortical and amygdala synaptosomes of rats that had received prolonged fluoxetine or sertraline treatment, as well a decrease in maximal [^{3}H]5-HT uptake in cortical and hippocampal slices obtained from rats that had received paroxetine for 21 days (Hrdina, 1987; Butler et al., 1988; Piñeyro et al., 1994). In rats that had received citalopram administered in their diets, no functional changes were observed when assessed in whole-brain synaptosomes (Hyttel et al., 1984). Sustained administration of sertraline and paroxetine decreases the number of SERTs in amygdala, perirhinal cortex, hippocampus, and rat frontal cortex (Kovachich et al., 1992; Piñeyro et al., 1994), but fluoxetine induced an increase in [^{3}H]paroxetine binding in the two latter

areas (Hrdina and Vu, 1993). However, at the same dose as in the last study and using the same route of administration, fluoxetine was seen to decrease V_{max} in cortical synaptosomes (Hrdina, 1987). Since synaptosomes in which V_{max} was decreased were prepared from frontal cortex, and the increased numbers in SERT sites was observed in frontoparietal, striatal, and hippocampal cortices, a possible explanation for these two sets of divergent observations following fluoxetine treatment could be accounted for by regional differences in the adaptative properties of the SERTs. Hybridization studies indicate a decrease in SERT mRNA in midbrain raphe nuclei after prolonged fluoxetine administration via osmotic minipumps (Lesch et al., 1993). Whether this decrease in transcriptional activity is secondary to a decrease in SERT protein turnover (in agreement with at least a transitory increase in the number of uptake sites), or it is the cause for a reduction in SERT number, cannot be deduced from these results. Once again, studies in which prolonged SSRI administration induced no changes in SERT activity or binding sites were performed in homogenates and/or drugs were administered i.p. (Graham et al., 1987; Kovachich et al., 1992; Dewar et al., 1993; Spurlock et al., 1994). The fact that, in spite of both sertraline and citalopram being administered i.p., only the first produced a reduction in [^{3}H]cyanoimipramine binding (Kovachich et al., 1992), and cortical 5-HT V_{max} (Butler et al., 1988) is probably due to the fact that, of these two SSRIs, only sertraline has an active metabolite whereas citalopram is rapidly inactivated (see Piñeyro et al., 1994). Finally, results from prolonged tricyclic administration may not be systematized: imipramine and desipramine have been shown to decrease [^{3}H]imipramine but not [^{3}H]paroxetine binding whereas chlomipramine, not only a tricyclic but also a potent and selective 5-HT uptake blocker, did not produce any significant change in SERT sites or its mRNA (Table 9). In a recent study by our group, three main lines of evidence indicate a reduction in 5-HT uptake activity in the rat hippocampus after prolonged administration of the SSRI paroxetine: 1) a decrease in the density of SERT sites, 2) a tolerance to the in vivo electrophysiological effects of the drug, and 3) a decrease of the in vitro [^{3}H]5-HT uptake capacity. A reduction in the total number of [^{3}H]paroxetine-binding sites in cortical and hippocampal membranes and a decrease in the amount of [^{3}H]5-HT taken up by dorsal raphe slices indicate that the plasticity of the SERT occurs not only in multiple projection areas, but also in the cell body and dendrites of 5-HT neurons (Piñeyro et al., 1994). Superfusion experiments in midbrain raphe slices provide additional evidence of the functional consequences of somatodendritic SERT down-regulation: after prolonged administration of paroxetine, the electrically evoked release of [^{3}H]5-HT in rat and guinea pig midbrain raphe slices is much higher than that observed after sustained befloxatone treatment (El Mansari and Blier, 1996; Piñeyro and Blier, 1996). That the enhanced somatodendritic output of [^{3}H]5-HT is due to SERT desensitization was demonstrated by the fact that superfusion of midbrain raphe slices with medium containing 1 μM paroxetine (introduced 20 min before the stimulations) produced a 50% increase in slices obtained from saline- or befloxatone-treated rats but remained unchanged in slices obtained from rats that had received paroxetine for 21 days. Furthermore, in the frontal cortex of long-term paroxetine-treated guinea-pigs, there is no 5-HT$_{1D}$ autoreceptor desensitization (unlike in the hippocampus and the hypothalamus), and electrically evoked [^{3}H]5-HT release is still enhanced (Blier and Bouchard, 1994; El Mansari and Blier, 1996). This is due to a desensitization of the SERT that was demonstrated by the reduced effectiveness of the same dose of paroxetine in enhancing [^{3}H]5-HT uptake in frontal cortex slices of guinea pigs treated with the SSRI for 21 days, as compared to 2 days (Blier and Bouchard, 1994).

Based on the previous analysis, the observations in Table 9 could be summarized by saying that an increase in 5-HT uptake may be expected after repeated ECS or sustained MAOI administration, whereas a decrease in this function is more likely to occur after prolonged SSRI treatment. Hence, it seems that the antidepressant effect is not correlated with a specific adaptative response of the 5-HT carrier.

Apart from drugs, numerous physiological processes regulate 5-HT uptake activity. Understanding them and finding the similarities they might have with any given pharmacological treatment may help us to understand how antidepressant drugs or ECS regulate SERT activity. ACTH and ACTH fragments up-regulate SERT expression during 5-HT neuron differentiation (Azmitia and De Kloet, 1987; Eaton and Whittemore, 1990). A transcription increase also occurs in raphe neurons of aged rats, an effect that may compensate for 5-HT leakage from degenerating terminals and/or increased release induced by age-related decline in terminal autoreceptor regulation (Meister et al., 1995). Similarly, 5-HT neuron sprouting in raphe nuclei following a 5,7-DHT lesion has been correlated with an increase in [^{3}H]paroxetine B_{max} values in the brainstem (Pranzatelli and Martens, 1992). Up-regulation of the SERT secondary to transcriptional activation may occur via an increase in cAMP (Cool et al., 1991; King et al., 1992; Ramamoorthy et al., 1993a) which has been recently proposed to induce the activation of the hSERT gene promoter via immediate early gene products such as transcription factors of the c-fos/c-jun family (Heils et al., 1995). cAMP-independent mechanisms activated by interleukin-1β (Ramamoorthy et al., 1995b) or the PKC inhibitor staurosporine (Ramamoorthy et al., 1995a) may also activate SERT mRNA production. Alternatively, 5-HT uptake activity may be rapidly enhanced without altering the transporter density by a mechanism involving transporter phosphorylation/dephosphorylation. Nitric oxide-

cGMP pathway activation has been shown to have this effect (Miller and Hoffman, 1994). Phosphorylation/dephosphorylation of the transporter via other signaling pathways such as PKC and calmodulin induce an opposite effect, reducing 5-HT uptake (Myers et al., 1989; Anderson and Horne, 1992; Jayanthi et al., 1994). It is unlikely, however, that these rapid regulatory responses may account for the decrease in 5-HT uptake observed after prolonged SSRI administration, since the former take place within 1 h or less after treatment, and in the case of paroxetine, tolerance to the drug was not expressed within a 48-h period but rather after a various number of days of treatment (Piñeyro et al., 1994). Furthermore, a recent study indicating that reduction in SERT mRNA does not always result in a decrease in the number of SERT in 5-HT neurons (Yu et al., 1994) suggests that a decrease in transcription might not always serve as an explanation for a long-latency down-regulation in the SERT. On the other hand, a dysregulated expression of the *SERT* gene (Heils et al., 1995) has been suggested as a possible explanation for one of the most consistent findings in biological psychiatry: the disease-associated decrease in brain and platelet 5-HT uptake sites observed in patients with affective disorders (see Lesch and Bengel, 1995).

IV. Concluding Remarks

This article provided an overview of the morphological aspects of the 5-HT system, analyzed the autoregulatory mechanisms with which 5-HT neurons are endowed and how they contribute to the regulation of firing activity, neurotransmitter release, and reuptake, along with the drugs that modify these functions. Special attention has been given to antidepressant drug treatments, their long-term effects, and possible strategies that may allow one to bypass this complex autoregulatory machinery to achieve quicker and/or more effective antidepressant responses. Autoregulatory processes not previously considered as targets for antidepressant drugs, such as regulation of somatodendritic 5-HT release by non-5-HT_{1A} receptors, have also been considered. Although many of the mechanisms described may still be a matter of debate, there is no doubt that a comprehensive approach to the physiology of the 5-HT system is paving the road to a better pharmacological management of affective and anxiety disorders.

Acknowledgments. We thank Helen Cameron and Gilda Corpadean for excellent secretarial assistance and Professors E. Azmitia, L. Descarries, P. Hrdina, and E. Hamel for helpful comments and discussion during the preparation of the manuscript. P.B. was a scientist of the Medical Research Council of Canada and G.P. was a Medical Research Council fellowship recipient.

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