The Pharmacology of the Neurochemical Transmission in the Midbrain Raphe Nuclei of the Rat

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Abstract: Midbrain slices containing the dorsal and medial raphe nuclei were prepared from rat brain, loaded with [^{3}H]serotonin ([^{3}H]5-HT), superfused and the release of [^{3}H]5-HT was determined at rest and in response to electrical stimulation. Compartmental analysis of [^{3}H]5-HT taken up by raphe tissue indicated various pools where the neurotransmitter release may originate from these stores differed both in size and rate constant. 5-HT release originates not only from vesicles but also from cytoplasmic stores *via* a transporter-dependent exchange process establishing synaptic and non-synaptic neurochemical transmission in the serotonergic somatodendritic area. Manipulation of 5-HT transporter function modulates extracellular 5-HT concentrations in the raphe nuclei: of the SSRIs, fluoxetine was found 5-HT releaser, whereas citalopram did not exhibit this effect. Serotonergic projection neurons in the raphe nuclei possess inhibitory 5-HT_{1A} and 5-$HT_{1B/1D}$ receptors and facilitatory 5-HT_3 receptors, which regulate 5-HT release in an opposing fashion. This observation indicates that somatodendritic 5-HT release in the raphe nuclei is under the control of several 5-HT homoreceptors. 5-HT_7 receptors located on glutamatergic axon terminals indirectly inhibit 5-HT release by reducing glutamatergic facilitation of serotonergic projection neurons. An opposite regulation of glutamatergic axon terminals was also found by involvement of the inhibitory 5-HT_7 and the stimulatory 5-HT_2 receptors as these receptors inhibit and stimulate glutamate release in raphe slice preparation, respectively, Furthermore, postsynaptic 5-$HT_{1B/1D}$ heteroreceptors interact with release of GABA in inhibitory fashion in raphe GABAergic interneurons. Serotonergic projection neurons also possess glutamate and GABA heteroreceptors; NMDA and AMPA receptors release 5-HT, whereas both $GABA_A$ and $GABA_B$ receptors inhibit somatodendritic 5-HT release. Evidence was found for reciprocal interactions between serotonergic and glutamatergic as well as serotonergic and GABAergic innervations in the raphe nuclei. Serotonergic neurons in the raphe nuclei also receive noradrenergic innervation arising from the locus coeruleus and alpha-1 and alpha-2 adrenoceptors inhibited [^{3}H]5-HT release in our experimental conditions. The close relation between 5-HT transporter and release-mediating 5-HT autoreceptors was also shown by addition of L-deprenyl, a drug possessing inhibition of type B monoamine oxidase and 5-HT reuptake. L-Deprenyl selectively desensitizes 5-HT_{1B} but not 5-HT_{1A} receptors and these effects are not related to inhibition of 5-HT metabolism but rather to inhibition of 5-HT transporter.

Key Words: Raphe nuclei, brain slices, [^{3}H]5-HT release, 5-HT receptors, serotonergic projection neurons, neurotransmitter interaction, monoamine oxidase.

1. INTRODUCTION

Several lines of evidence indicate since the early discovery of Dahlstom and Fuxe [48] that the cell bodies of serotonergic neurons are concentrated in the midbrain areas. Of the serotonin (5-HT) cell groups present in the brainstem, the dorsal and median raphe nuclei have been studied most frequently. At least 50% of all serotonergic neurons of the central nervous system are located in the dorsal raphe nucleus [175], which endows an important interface function with its extensive afferent and efferent connections [126]. Neurotransmitter systems often interact in a reciprocal fashion in the central nervous system. This may also be true for the raphe nuclei in which substantial neural elements, the medium-sized serotonergic neurons, the GABAergic interneurons and the glutamatergic axon terminals modulate each other's function [118, 119]. The aim of this review was to overview auto- and heteroregulation of serotonergic projection neurons by various pharmacological agents, and also to discuss possible reciprocal interactions among the main neurotransmitter systems within the raphe nuclei.

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1.1. Serotonergic Projection Neurons

Almost 70% of the cells in the midbrain raphe nuclei are serotonergic [176]. Most of the serotonergic neurons are medium sized cells with spiny dendritic arborization, although serotonin cells with various morphological features were also observed in the dorsal raphe nucleus [2, 139]. Serotonergic presynaptic dendrites are synapsed upon other 5-HT-containing dendrites establishing a local regulation of 5-HT neurons within the raphe nuclei [92, 176]. Besides dendrodendritic interactions, it has been suggested that the activity of serotonergic neurons may be regulated locally by recurrent axon collaterals [73]. Serotonergic collaterals may be intrinsic to the raphe nuclei but 5-HT terminals may also originate from other 5-HT raphe cell groups. Serotonergic interconnections among various raphe nuclei have been demonstrated and they serve as a serotonergic input between the median and dorsal raphe nuclei [43]. 5-HT itself could regulate the activity of 5-HT neurons since synaptic afferents found on 5-HT dendrites and cell bodies are often serotonergic. The number of serotonergic nerve endings with synaptic specializations is however, is low, suggesting that the majority of 5-HT is released in a non-synaptic manner and thus, most of the neurons in the raphe nuclei are not under transsynaptic 5-HT control [73].

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Serotonergic neurons in the midbrain raphe nuclei exhibit spontaneous discharge activity of 1 to 5 spikes per second [163]. The activity of serotonergic neurons in the raphe nuclei is determined by at least three different events. These are the spontaneous, slow firing activity of 5-HT neurons, the 5-HT release-mediated autoinhibition of the neurons and the excitatory and inhibitory afferent inputs mediated by non-5-HT receptors [126]. The autoinhibitory properties of serotonergic neurons, which include regulation of transmitter release and synthesis and neural firing, are determined by various metabotropic (5-$HT_{1A/1B/1D}$, 5-$HT_{2A/2C}$, 5-HT_7) and ionotropic (5-HT_3) 5-HT receptors [11, 15, 180]. Different 5-HT_1 receptor subtypes are present on 5-HT-containing neurons in the raphe nuclei and they inhibit 5-HT release [49, 125, 149, 153] albeit their regulatory function in this process is different. In the raphe nuclei, few data are available about the types of 5-HT receptors mediating heteroreceptor functions i.e. those located on other neurons. Postsynaptic 5-HT receptors are the target of 5-HT released from dendritic varicosities into the synaptic gap or into the extracellular space as both synaptic and non-synaptic 5-HTergic neurotransmission may occur in the raphe nuclei [43, 73].

The release of 5-HT in the raphe nuclei originates from the somatodendritic area, the recurrent axon collaterals or axon terminals and the regulation of 5-HT release from these sites may be different. Dendritic 5-HT release originates from serotonergic neurons with vesicles within the dendrites [82], which then may provide neurochemical basis for synchronous firing pattern observed among dorsal raphe serotonergic cells. A further piece of evidence for the presence of 5-HT receptors on serotonergic neurons is that iontophoretically applied 5-HT decreases the firing of raphe neurons. Besides, 5-HT released from 5-HT-containing neurons, the repetitive cycles of hyper- and depolarization of 5-HT-containing cells are altered by heteroregulation of excitatory and inhibitory projection neurons and interneurons [125].

1.2. GABAergic Neurons in the Raphe Nuclei

A part of non-5-HT neurons in the raphe nuclei are γ-aminobutyric acid (GABA)-containing interneurons [20] and they participate in GABAergic-serotonergic interaction in this brain region [73, 174]. These GABAergic interneurons establish a local network in the dorsal raphe nucleus making synaptic connections with neurons containing 5-HT [104, 112]. GABAergic interneurons innervate large principal cell populations at the perisomatic region or at their dendrites inducing either synchronization or control of glutamatergic inputs. There is morphological as well as neurochemical evidence for the GABAergic-serotonergic interaction in the raphe nuclei [62, 73, 105]. GABA inhibits serotonergic system as it decreases the turnover rate of 5-HT in the median raphe nucleus [62]. In addition, serotonergic neural activity was blocked by local application of GABA agonists in the raphe nuclei [64] and both $GABA_A$ and $GABA_B$ receptors may be involved in the GABAergic inhibitory influence on 5-HT cells [158]. Moreover, $GABA_A$ and $GABA_B$ receptor subtypes located on presynaptic axon terminals of GABAergic neurons possess release-mediating autoreceptor functions [98]. GABAergic inhibition may also be mediated by efferent projections and the fibers of the habenulo-raphe pathway are one of the possible sources for this influence [155, 166, 173]. The periaqueductal gray matter as well as the amygdala may also be sources of GABAergic neurons projecting to the dorsal raphe nucleus [1, 20, 88]. GABAergic efferents may also terminate GABA interneurons in the raphe nuclei [73]. GABAergic-GABAergic neural interactions frequently occur in the central nervous system and this interaction may be mediated by postsynaptic $GABA_A$ receptors. The importance of GABAergic-GABAergic neural connection is to establish feed-forward inhibition [95].

1.3. External Connections of the Raphe Nuclei with Other Brain Structures, Afferent and Efferent Pathways

Conversion of incoming signals into output messages occurs in the internal neural circuitry of the raphe nuclei. Neural interactions operating in a reciprocal fashion establish a fine tune between the major cells types in the raphe nuclei. The neural network of serotonergic projection neurons, GABAergic interneurons and glutamatergic axon terminals forms complicated excitatory-inhibitory connections by which incoming excitatory signals are converted into inhibitory output and sent back to brain areas from where stimulation was generated. The postulated neural interactions are supported by morphological findings. The functional state of this internal neural network is determined by various neurotransmitters released. The actual concentrations of neurotransmitters in the synaptic clefts or in the biophase are regulated by receptors, transporters and also by enzymes involved in metabolism of transmitter molecules, such as monoamine oxidases (MAOs), in case of biogenic amines [97]. Both synaptic and non-synaptic release of neurotransmitters [170] might be involved in the local 5-HTergic, GABAergic and glutamatergic circuitry of the raphe nuclei.

The ascending projections of raphe serotonergic neurons influence several central nervous functions in different forebrain structures [121, 154]. *In vivo* electrophysiological experiments indicate that activation of ascending serotonerg pathway causes a poststimulus inhibition of 5-HT cell firing in the dorsal raphe nucleus [71]. This effect is mediated through 5-HT released from dendrites and axon varicosities within the raphe nuclei. 5-HT release can be demonstrated from both somatodendritic and axon terminal parts of these neurons, although the two areas show several differences in 5-HT content, turnover rate, and receptor density [8]. The function of 5-HT autoreceptors located on somatodendritic and axon terminal areas is also different: the former regulates dendritic 5-HT release and action potential generation, and the latter primarily governs impulse-mediated 5-HT release of vesicular origin [180].

The raphe-hippocampal pathway is one of the numerous ascending serotonergic projections in the brain [55]. The caudate nucleus also represents an important projection field for serotonergic neurons located in the raphe nuclei [162, 168]. Serotonergic neurons of the raphe nuclei form extended projections to the cerebral cortex [71, 94, 146] and the raphe-cortical 5-HT neurons mediate an inhibition in the cortical neural network [116, 154]. In many cases, forebrain structures that receive serotonergic innervations from the raphe nuclei send neuronal inputs back to the midbrain serotonergic nuclei [165]. Morphological evidence has been presented for the existence of a glutamatergic pathway arising

from the median prefrontal cortex and projecting to the raphe nuclei [16]. Furthermore, it has been shown that GABA interneurons in the raphe nuclei are the primary targets for the cortico-raphe glutamatergic neurons [42] and their stimulation by glutamate release leads to inhibition of 5-HTergic cells [71, 165]. Serotonergic neurons in the raphe nuclei are regulated by a number of efferent projections. Thus, afferents to the dorsal raphe nucleus originate from the substantia nigra [91, 155] or from the hypothalamus [103]. The habenulo-raphe projection represents a major GABAergic inhibition to the raphe nuclei cells [5, 155, 174]. The habenulo-raphe pathway also contains excitatory transmitters and the presence of substance P- and glutamate-containing fibers was reported. Another neural pathway that projects onto the raphe serotonergic neurons is noradrenergic and mediates its effect through postsynaptically located α-adrenoceptors [13, 23, 124]. Noradrenergic projections for the dorsal and median raphe nuclei arise from the locus coerulus and the lateral tegmental area, respectively [111, 138]. Hopwood and Stamford [84] have reported that both alpha-1 and alpha-2 adrenoceptors are involved in inhibition of stimulated 5-HT release from rat dorsal and median raphe nuclei slices.

2. CHARACTERIZATION OF SOMATODENDRITIC [^{3}H]5-HT RELEASE

2.1. Measurement of [^{3}H]5-HT Release from Raphe Nuclei Slices

Midbrain slices containing the raphe nuclei can be prepared from rat brain for determination of somatodendritic [^{3}H]5-HT release [93]. Slices approximately 350 μm thick are cut from the dissected tissue using a McIlwain tissue chopper (Fig. **1**) and are collected in Krebs-bicarbonate buffer. Brain slices containing the raphe nuclei are then incubated with [^{3}H]5-HT dissolved in Krebs-bicarbonate buffer for 30 min at 36 °C [9]. At the end of the loading period, the tissues are transferred into low volume superfusion chambers [90] and superfused with aerated (95% O_2/5% CO_2) and preheated (37 °C) Krebs-bicarbonate buffer by using a peristaltic pump. The superfusate is discarded for the first 60-min period of the experiments then a series of 3-min fractions is collected by a fraction collector. Biphasic electrical field stimuli (20 to 40 V voltage, 2 Hz frequency, 2-msec impulse duration for 2 min) are delivered in fractions 4 and 15 by an electrostimulator to stimulate the efflux of [^{3}H]5-HT from superfused raphe nuclei slices. Agonists are added to the superfusion buffer 9 min and antagonists are added 18 min before the 2nd electrical stimulation of the slices and drugs are maintained throughout the experiment. At the end of the experiments, the tissues are collected from the superfusion chambers and solubilized. The radioactivity released from the tissue and that remained in the tissue is determined by liquid scintillation spectrometry. In order to determine [^{3}H] norepinephrine (NE) release, raphe unclei slices were loaded with [^{3}H] NE in conditions described above.

When the release of amino acids from raphe nuclei slices is measured, the slices are incubated with either [^{3}H]glutamate or [^{3}H]GABA dissolved in aerated Krebs-bicarbonate buffer at 36 °C for 30 min. For [^{3}H]GABA release, raphe nuclei slices are loaded in the presence of β-alanine, an inhibitor of GABA uptake in glial cells, and superfused with a buffer containing the aminotransferase inhibitor aminooxyacetic acid and the GABA uptake inhibitor nipecotic acid [76].

2.2. Characterization of [^{3}H]5-HT Release from Raphe Nuclei Slices

The time course of [^{3}H]5-HT release measured from raphe nuclei slices of the rat is shown in Fig. (**2**). The efflux of [^{3}H]5-HT is expressed as Bq/g/3 min fraction or as a fractional rate, i. e. as a percentage of the amount of [^{3}H]5-HT in the tissue at the time of the release is determined [75]. The fractional rate of [^{3}H]5-HT efflux obtained for the first (S1) and second (S2) electrical or KCl-induced stimulations and the ratio of S2/S1 are calculated to determine the effects of drugs on [^{3}H]5-HT release. The radioactivity released from raphe nuclei slices preloaded with [^{3}H]5-HT can be separated into [^{3}H]5-HT and [^{3}H]metabolites on Sephadex G-10 resin [142]. Similar experimental procedure can be applied for [^{3}H]GABA release although the fractional rate of the release is small compared to [^{3}H]5-HT release [12]. Glutamate stores in axon terminals exhibit fast exhaustion and release can be elicited only with one electrical stimulation in contrast to 5-HT and GABA, for these neurotransmitters, repeated electrical or chemical stimulation could be carried out [12, 80].

The release of [^{3}H]5-HT determined from raphe nuclei slices was characterized by the resting and the electrical stimulation-induced release and they were compared to those measured from hippocampal slices, an area containing axon terminal parts of serotonergic neurons. The resting release was 2.04±0.13 and 2.00±0.05% of content released in 3 min in the raphe and hippocampal slices, the electrically evoked release was 4.32±0.13 and 5.84±0.22% of content released in

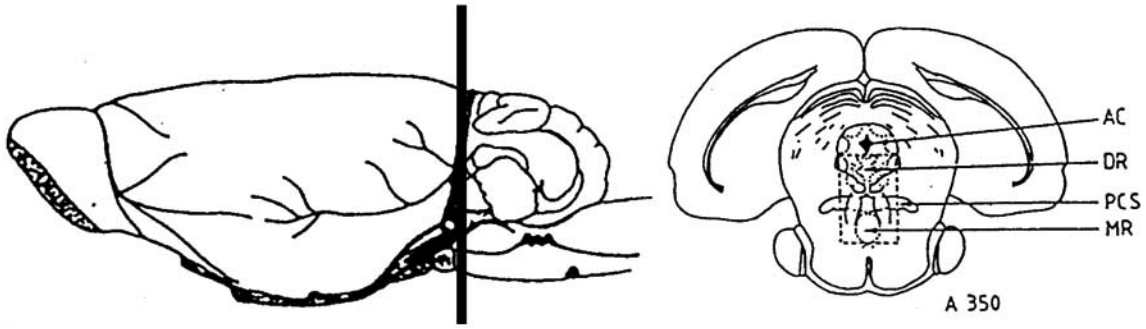

Fig. (1). Preparation of raphe nuclei slice from rat brain. AC, cerebral aqueduct; DR, dorsal raphe; MR, median raphe; PCS, superior cerebellar peduncle. Vertical line indicates direction of brain transection.

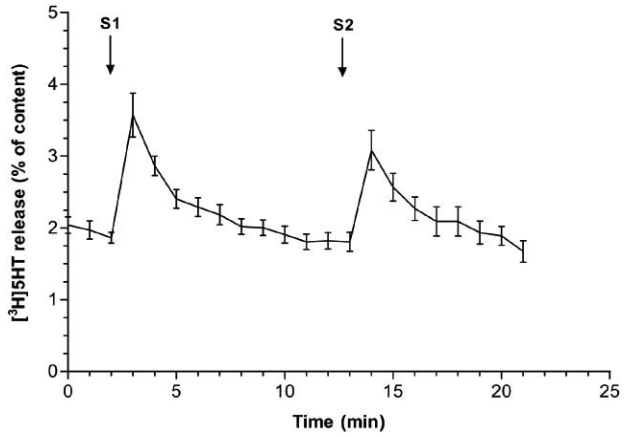

Fig. (2). The time course of [^{3}H]5-HT release measured from raphe nuclei slices of the rat. Slices containing the raphe nuclei were prepared from rat brain, loaded with [^{3}H]5-HT and superfused with Krebs-bicarbonate buffer. The slices were stimulated electrically (20 V, 2 Hz, 2-msec for 2 min in fractions 4(S1) and 15(S2) and the fractional release of [^{3}H]5-HT (i. e. a percentage of the amount of [^{3}H]5-HT in the tissue at the time of the release) was calculated by a computer program. The net fractional release (evoked release minus resting release) was 3.08% of [^{3}H]5-HT content released for the 1st stimulation (S1), this value was 2.73% for the 2nd (S2) stimulations. The calculated ratio of fractional release S2 over fractional release S1 (S2/S1) was 0.89. Mean±S.E.M., n=5.

3-min fraction in these brain areas, respectively. Although the resting and the evoked [^{3}H]5-HT release did not differ in raphe nuclei and hippocampus the net fractional release (evoked release minus resting release) was 3.67±0.42 and 7.59±0.42% of content released per stimulus in the central and peripheral parts of serotonergic neurons. These findings indicate differences between the size of storage and release pools for 5-HT in somatodendritic and axon terminal fields.

2.3. Compartmentalization of [^{3}H]5-HT Taken Up into Raphe Nuclei Slices

Neurotransmitter storage in and efflux from raphe nuclei tissue can be characterized by compartmental analysis of the radioactivity taken up during loading period. The pools of [^{3}H]5-HT vary in their rate constant, turnover rate, and turnover time. In slices containing the raphe nuclei, accumulated [^{3}H]5-HT distributes mainly in two compartments: an efflux compartment from where release of [^{3}H]5-HT occurs and a bound fraction (slowly exchanging compartment), which does not contribute to the efflux [51]. The size of bound fraction is estimated by plotting the rate of [^{3}H]5-HT efflux in function of radioactivity remaining in the tissue. The desaturation curve obtained in the de Langer-Mulder plot is linear and the intercept of the regression line with the abscissa estimates the size of the bound fraction. Substraction of the bound fraction from the tissue content of radioactivity, determined at the beginning of superfusion, reveals the efflux compartment.

Using the de Langer-Mulder plot, the size of the bound and the efflux compartments was calculated for [^{3}H]5-HT in the raphe nuclei. This analysis revealed that at about 76.21±3.19% of the total amount of [^{3}H]5-HT taken up represent the efflux compartment and the remaining 23.77±3.19% contribute to the bound fraction. It can also be determined that at about 39% of radioactivity stored in the efflux compartment is released during a 66-min superfusion period. Moreover, compartmental analysis of the radioactivity stores in raphe tissue also indicates that the efflux compartment consists of various pools for [^{3}H]5-HT in the raphe nuclei. Desaturation curve for each compartment can be determined from the de Langer-Mulder plot and the intercept of the regression line with the abscissa estimates the size of the pool that participates in the release. The involvement of different efflux compartments of [^{3}H]5-HT in the release process was also shown as the efflux of [^{3}H]5-HT was plotted in function of time (Fig. **3**). As shown in Fig. (**3**), the efflux compartment can be divided into at least three different pools from which fast and slow efflux of [^{3}H]5-HT occur in response to electrical stimulation and a third one which represents the source of resting outflow of [^{3}H]5-HT.

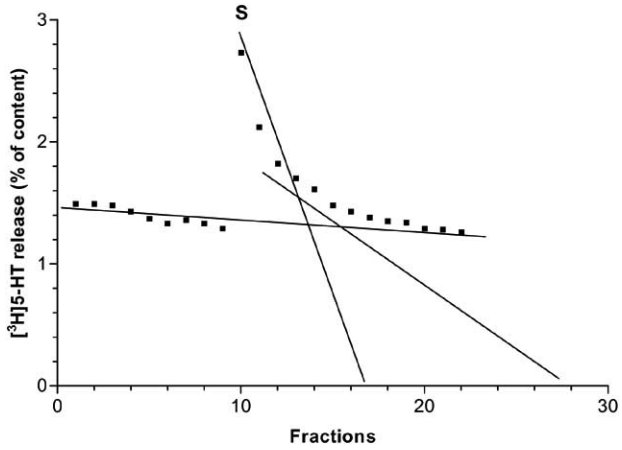

Fig. (3). Release of [^{3}H]5-HT from raphe nuclei slices, a compartmental analysis. The fractional release of [^{3}H]5-HT was plotted in function of superfusion time. The efflux of [^{3}H] 5-HT occurs with different fractional rate constants indicating involvement of more than one compartment in the release process. Distributional analysis revealed that the participation of the fast, slow and resting release occurs from pools representing 16.35±1.48, 28.21±2.96 and 55.42±4.41% of the efflux compartment, respectively. The slices were stimulated (S) electrically (20 V, 2 Hz, 2-msec for 2 min) in fraction 10. For experimental procedure see Fig. (**2**).

2.4. The Role of 5-HT Transporter in the Somatodendritic [^{3}H]5-HT Release Process

The fact, that 5-HT transporters are operative even *in vitro* in superfused tissue, was demonstrated in raphe nuclei slices superfused with Krebs-bicarbonate buffer containing selective 5-HT reuptake inhibitors. As shown in Fig. (**4A**), fluoxetine increased both resting and electrical stimulation-induced [^{3}H]5-HT release. This finding indicates that fluoxetine is not only a 5-HT uptake inhibitor but also releases 5-HT from dendrites or nerve terminals. In contrast, the 5-HT uptake inhibitor citalopram [81] failed to induce any potentiation on resting or electrically evoked [^{3}H]5-HT release in superfused raphe nuclei slices, indicating uptake inhibitory but not release properties of this compound (Fig. **4B**).

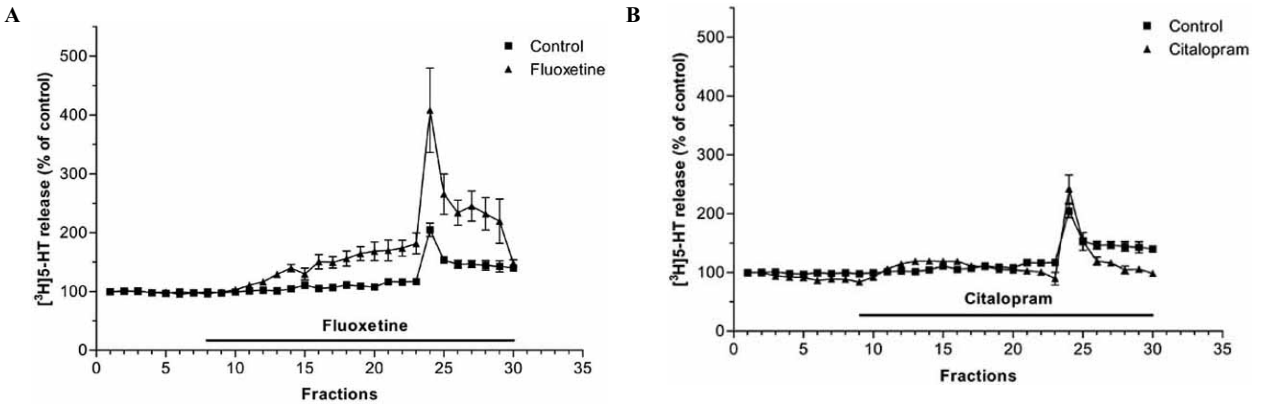

Fig. (4). Effect of selective serotonin reuptake inhibitors on resting and electrically stimulated [^{3}H]5-HT release in raphe nuclei slices of the rat. Fluoxetine (A) and citalopram (B) were added in 10 and 30 μM concentrations from fraction 9 and maintained through the experiments. The slices were stimulated electrically (20 V, 2 Hz, 2-msec for 2 min) in fraction 24. The mean of the release in fractions 1, 2 and 3 was taken as 100%. For experimental procedure see Fig. **2**. Mean±S.E.M., n=3.

Interaction between neuronal transporters and presynaptic release-mediating autoreceptors was already reported [99, 122]. 5-HT uptake inhibitors have been shown to antagonize the inhibitory effect of LSD on the electrically evoked overflow of [^{3}H]5-HT from rat hypothalamic slices [65]. Moreover, 5-HT$_{1A}$ autoreceptors are desensitized in the dorsal raphe nucleus of knockout mice lacking the 5-HT transporter [21]. Such interaction between autoreceptor and transporter may have importance in the mechanism on how L-deprenyl desensitizes 5-HT$_{1B}$ receptors [77]. The close functional connection between neurotransmitter transporters and presynaptic autoreceptors is supported by the findings that repeated administration of 5-HT reuptake blockers alters sensitivity of release-mediating 5-HT$_1$ receptor subtypes in the somatodendritic area as well as in the axon terminals [17, 126]. In the dorsal raphe nucleus of the rat, the 5-HT$_{1A}$ receptor partial agonist pindolol attenuated the inhibitory effect of fluoxetine on 5-HT cell firing [130]. Another piece of evidence for receptor-transporter functional interaction is shown by our finding that the 5-HT$_{2A/2C}$ antagonist deramciclane potentiates the 5-HT uptake inhibitory effect of fluoxetine in raphe nuclei slices (unpublished observation). This finding is in line of previous suggestions that 5-HT$_2$ receptor antagonists may augment 5-HT uptake inhibitory properties of certain compounds [36].

3. 5-HT RECEPTOR-MEDIATED 5-HT RELEASE IN RAPHE NUCLEI SLICES

3.1. 5-HT$_1$ Receptor-Mediated 5-HT Release in the Raphe Nuclei: Feedback Inhibition

The somatodendritic 5-HT$_1$ receptors have multifunctional roles since they are involved in the regulation of 5-HT release and synthesis as well as in the regulation of neuronal impulse generation and firing activity [7, 9, 49, 83]. Raphe nuclei possess at least two autoreceptor mechanisms that control 5-HT release: one directly mediated by 5-HT$_{1B}$ and/or 5-HT$_{1D}$ receptors and the other one indirectly mediated by 5-HT$_{1A}$ autoreceptors that also modulate neural firing rate [15]. The release inhibitory 5-HT$_{1B}$ receptors are situated on recurrent axon collaterals and axon terminals within the raphe nuclei [4, 49, 55, 56], these receptors exert small effect on the firing of these neurons [136]. Apart, 5-HT$_{1B}$ autoreceptors, inhibition of 5-HT$_{1D}$ receptor-mediated 5-HT releases in the raphe nuclei is also independent from regulation of neuronal firing [47]. 5-HT$_{1D}$ receptors are autoreceptors in the raphe nuclei and may control larger somatodendritic 5-HT release [150]. Moreover, changes in 5-HT neuronal firing activity are the consequence of 5-HT$_{1A}$ receptor activation and firing-dependent control of 5-HT release occurs in the cell body level mediated by 5-HT$_{1A}$ receptors [83]. The release-inhibitory 5-HT$_{1A}$ receptors are located on the somatodendritic part of 5-HT neurons and control the bulky, possibly non-synaptic release of 5-HT [115, 117]. It has been suggested that the multiple 5-HT$_1$ receptor-mediated mechanisms, that control 5-HT function in the raphe nuclei, open new therapeutic possibilities for psychiatric disorders [151]. Using midbrain slice preparations containing raphe nuclei, it was possible to demonstrate the inhibitory effects of various 5-HT$_1$ receptor subtypes on 5-HT release *in vitro* [9].

3.1.1. Effects of Agonists

The 5-HT$_{1A}$ receptor agonist 8-OH-DPAT [109] inhibited the electrical stimulation-induced [^{3}H]5-HT release from midbrain raphe slices of rats and this inhibition proved to be concentration dependent (Fig. **5**). 8-OH-DPAT did not affect the spontaneous release of [^{3}H]5-HT. In contrast to its effect on raphe nuclei tissue, 8-OH-DPAT failed to alter the electrical stimulation-induced [^{3}H]5-HT release from superfused hippocampal slice preparations, a brain area containing serotonergic axon terminals (Table **1**). Our results are further indication that the somatodendritic 5-HT autoreceptors in the raphe nuclei belong to 5-HT$_{1A}$ receptor subtypes [167], whereas 5-HT$_{1A}$ receptors present in the hippocampus do not serve as release-regulating presynaptic homologous autoreceptors.

The 5-HT$_{1B}$ receptor agonist CGS-12066 [113, 144] also inhibited electrically stimulated [^{3}H]5-HT release in raphe nuclei slices (Fig. **5**). Besides raphe nuclei slices, [^{3}H]5-HT release from hippocampal slices was also inhibited by CGS-

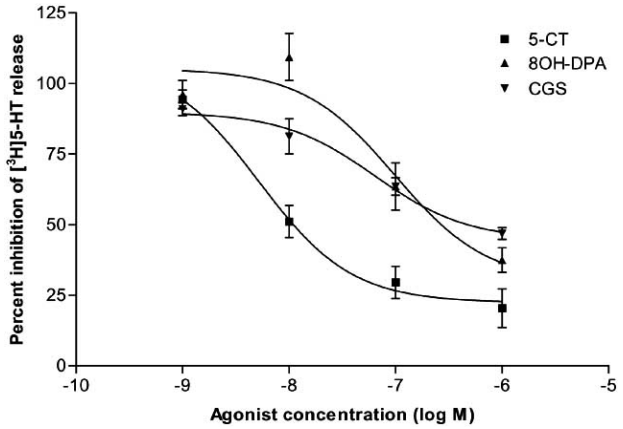

Fig. (5). Effects of 8-OH-DPAT, CGS-12066 and 5-CT on electrical stimulation-induced [^{3}H]5-HT release from raphe nuclei slices. The calculated IC_{50} values to inhibit [^{3}H]5-HT release were 0.01, 0.28, and 0.58 μM for 5-CT, 8-OH-DPAT, and CGS-12066, respectively. For experimental procedure see Fig. (**2**). Drugs were added to the tissue from fraction 10 and maintained through the experiment. Electrical stimulation-induced [^{3}H]5-HT release expressed as S2/S1 that was taken as 100% was 0.92±0.06. Mean±S.E.M., n=5.

12066 (Table **1**) further supporting the view that the release-mediating 5-HT receptors located at the nerve endings belong to 5-HT_{1B} receptor subtype [110]. These data also confirm that 5-HT_{1A} and 5-HT_{1B} receptors are present in the raphe nuclei and control somatodendritic as well as axon terminal release of 5-HT. It is not clear where these receptors are situated on serotonergic neurons although the location of 5-HT_{1A} receptors on the somatodendritic part and the location of 5-$HT_{1B/1D}$ receptors on recurrent axon collaterals have been suggested [49] and thus these might be the sites for the action of 8-OH-DPAT and CGS-12066, respectively.

5-Carboxamidotryptamine (5-CT), which has an agonist effect on 5-HT_1 receptor subtypes [32, 72, 171] and 5-HT_7 receptors [33, 85], also inhibited the electrically evoked [^{3}H]5-HT release (Fig. **5**) without influencing the resting [^{3}H]5-HT outflow from raphe nuclei slices of the rat. This effect of 5-CT can be seen in both the somatodendritic and the axon terminal fields of raphe 5-HT neurons (Table **1**). The calculated IC_{50} value for 5-CT, concentration that induced 50% decrease in [^{3}H]5-HT overflow in raphe nuclei slices, was 3.34±0.37 nM and the rank order of [^{3}H]5-HT release inhibition in raphe nuclei slices was found to be 5-CT > 8-OH-DPAT > CGS-12066 [80].

3.1.2. Effects of Antagonists

The 5-HT_{1A} receptor antagonists (+)WAY-100135 [44] and WAY-100635 [60] did not affect the [^{3}H]5-HT release on their own right (Table **2**). The 5-$HT_{1B/1D}$ receptor antagonist SB-216641 [128. 143] increased electrical stimulation induced [^{3}H]5-HT release per se in superfused raphe nuclei slices. It has been reported that the non-selective 5-HT_{1D} receptor antagonist methiothepine and the selective 5-HT_{1D} receptor antagonist GR127935 also increased [^{3}H]5-HT release by themselves in guinea-pig cerebral cortex slices [131].

The inhibitory effect of 8-OH-DPAT on [^{3}H]5-HT release from [^{3}H]5-HT-preloaded raphe nuclei slices was also measured in the presence of (+)WAY-100135. Fig. (**6**) shows that (+)WAY-100135 dose-dependently antagonized the inhibitory effect of 8-OH-DPAT on [^{3}H]5-HT release in raphe nuclei slices. WAY-100635 only slightly influenced the inhibitory effect of 5-CT on [^{3}H]5-HT overflow and the apparent pA_2 value for WAY-100635 against 5-CT was 4.99 (Fig. **7**). SB-216641, a selective 5-HT_{1B} receptor antagonist with approximately 25-fold selectivity over 5-HT_{1D} receptors [128], also produced a concentration-dependent rightward shift of the concentration-inhibition curve of 5-CT on [^{3}H]5-HT release with no alterations in the maximal 5-CT response (Fig. **7**). The calculated apparent pA_2 value for SB-216641 on 5-CT-induced inhibition of [^{3}H]5-HT overflow was 7.12. This finding is in accord of the general view that 5-HT_{1B} and 5-HT_{1D} receptors are inhibitory autoreceptors in the 5-HT-containing raphe nuclei neurons [150] although different regulatory role of the two receptor subtypes was suggested [153].

3.1.3. Mode of Action: the Role of K^+-Channels

The voltage-sensitive K^+-channel blocker 4-aminopyridine (4-AP) [136] increased electrical stimulation-induced [^{3}H]5-HT release from raphe nuclei slices of rat, the effect of 4-AP

Table 1. Effects of Various 5-HT Receptor Agonists on Electrical Stimulation-Induced [^{3}H]5-HT Release from Raphe Nuclei and Hippocampal Slices of the Rat

Compounds	Receptor	Concentration (μM)	[^{3}H]5-HT release (S2/S1)	
			Raphe nuclei	Hippocampus
Control			0.96±0.06	1.06±0.14
8-OH-DPAT	5-HT_{1A}	1	0.39±0.07*	0.91±0.07
CGS-12066	5-$HT_{1B/1D}$	1	0.61±0.08*	0.53±0.09*
5-CT	5-HT_{1A}/5-HT_7	0.1	0.26±0.06*	0.23±0.02*

Hippocampal or raphe nuclei slices were prepared from rat brain, loaded with [^{3}H]5-HT and superfused with Krebs-bicarbonate buffer. The slices were stimulated electrically (20 V, 2 Hz, 2-msec for 2 min) in fractions 4(S1) and 15(S2). The fractional release of [^{3}H]5-HT for S1 and S2 and the S2/S1 ratio were calculated. Drugs were added to the tissue from fraction 10. ANOVA and Dunnett's test, *P<0.05, mean±S.E.M., n=3-5.

Table 2. Effects of Various 5-HT Receptor Antagonists on Electrical Stimulation-Induced [^{3}H]5-HT Release from Raphe Nuclei Slices of the Rat

Compounds	Receptor	Concentration (μM)	[^{3}H]5-HT release (S2/S1)
Control	-	-	0.80±0.02
(+)WAY-100135	5-HT$_{1A}$	1	1.08±0.04
WAY-100635	5-HT$_{1A}$	10	0.90±0.06
NAN-190	5-HT$_{1A}$	10	1.02±0.08
SB-216641	5-HT$_{1B/1D}$	1	1.11±0.09*
Ondansetron	5-HT$_3$	1	0.95±0.07
SB-271046	5-HT$_6$	10	0.98±0.03
SB-258719	5-HT$_7$	10	0.86±0.10

Raphe nuclei slices were loaded with [^{3}H]5-HT, superfused, and stimulated electrically (20 V, 2 Hz, 2-msec for 2 min) in fractions 4(S1) and 15(S2) and the release of [^{3}H]5-HT was expressed by the S2/S1 ratio. Drugs were added to the tissue from fraction 9. ANOVA and Dunnett's test, *P<0.05, mean±S.E.M, n=3-8.

was concentration dependent [9]. This finding indicates that 4-AP-sensitive K^+-channels are involved not only in axon terminal transmitter release [70, 87] but also in that which originates from the somatodendritic areas of 5-HT-containing cells. In contrast, neither glibenclamide nor tolbutamide, drugs blocking ATP-dependent K^+ channels [45], affected the electrical stimulation-induced [^{3}H]5-HT release in isolated rat raphe nuclei slices (Table **3**), suggesting that the ATP-sensitive K^+ channels are apparently not involved in the regulation of somatodendritic [^{3}H]5-HT release.

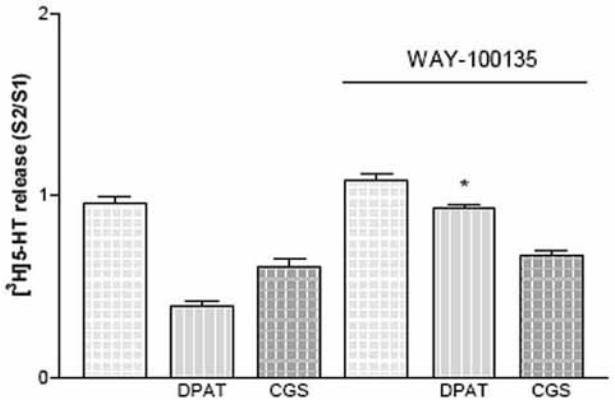

Fig. (6). WAY-100635 antagonized the inhibitory effect of 8-OH-DPAT on [^{3}H]5-HT release but it was ineffective on CGS-12066-induced inhibition in raphe nuclei slices of the rat. For experimental procedure see Fig. (**2**). 8-OH-DPAT (1 μM) and CGS-12066 (1 μM) were added 9 min and WAY-100135 (1 μM) was added 18 min before S2 and maintained through the experiment. ANOVA and Dunnett's test, *P<0.05, mean±S.E.M., n=3-4.

When 8-OH-DPAT or CGS-12066 was combined with different K^+ channel blockers, the inhibitory effect of 8-OH-DPAT on [^{3}H]5-HT release was abolished in the presence of 4-AP, whereas this effect of CGS-12066 was not influenced by the K^+ channel blocker (Table **3**). Glibenclamide, on the other hand, failed to affect the inhibitory effect of either 8-OH-DPAT or CGS-12066 on [^{3}H]5-HT release in raphe nuclei slices. This finding suggests that stimulation by 8-OH-DPAT of somatodendritic 5-HT$_{1A}$ autoreceptors opens voltage-sensitive K^+ channels in serotonergic neurons and the consequent hyperpolarization of the somatodendritic membrane may then cause a decreased [^{3}H]5-HT release [9]. It is possible, however, that stimulation of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors leads to opening of different K^+-channels as on the contrary to 5-HT$_{1A}$ receptors, the voltage-sensitive K^+ channels did not interfere with 5-HT$_{1B}$ receptor-mediated 5-HT release inhibition. In contrast to 4-AP, the ATP-sensitive K^+ channel inhibitor glibenclamide did not modify either 8-OH-DPAT- or CGS-12066A-induced inhibition of [^{3}H]5-HT release from raphe nuclei slices. Thus, the ATP-sensitive K^+

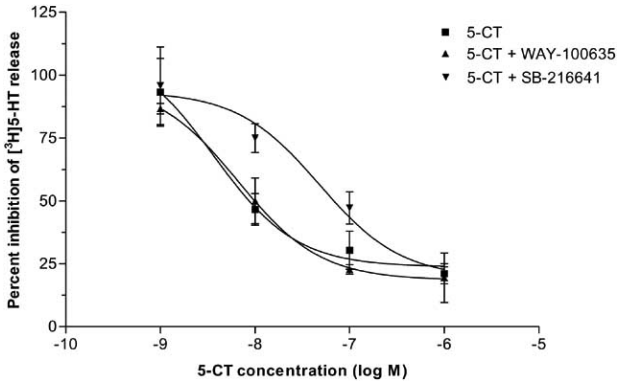

Fig. (7). Effects of WAY-100635 (10 μM) and SB-216641 (1 μM) on 5-CT-induced inhibition of [^{3}H]5-HT release in raphe nuclei slices of the rat. The IC$_{50}$ value of 5-CT to inhibit [^{3}H]5-HT overflow was 3.34±0.37 nM, it was 6.65±0.56 and 47.81±4.66 nM in the presence of WAY-100635 and SB-216641, respectively. For experimental procedure see Fig. (**5**). 5-CT was added 9 min and the 5-HT receptor antagonists were added 18 min before S2. Mean±S.E.M., n=3-4. (Figure taken from Ref. 80).

Table 3. Effects of Various K^+-Channel Inhibitors on 5-HT_1 Receptor Agonists-Induced [^{3}H]5-HT Release from Raphe Nuclei Slices of the Rat

Compounds	Concentration (μM)	[^{3}H]5-HT release (S2/S1)		
		Control	8-OH-DPAT	CGS-12066
None	-	0.99±0.06	0.42±0.04*	0.50±0.06*
4-Aminopyridine	50	1.91±0.33	2.19±0.26	0.87±0.16*
Glibenclamide	10	1.30±0.04	0.75±0.08*	0.43±0.09*
Tolbutamide	100	1.11±0.08	0.63±0.11*	-

Raphe nuclei slices were loaded with [^{3}H]5-HT, superfused, and stimulated electrically (20 V, 2 Hz, 2-msec for 2 min) in fractions 4(S1) and 15(S2) and the release of [^{3}H]5-HT was expressed by the S2/S1 ratio. Drugs were added to the tissue from fraction 9. 8-OH-DPAT and CGS-12066 were added in a concentration of 1 μM. ANOVA and Dunnett's test, *P<0.05, mean±S.E.M., n=3-8.

channels are probably not involved in the 5-HT_{1A} and 5-HT_{1B} receptor-mediated autoinhibition of somatodendritic 5-HT release [9].

3.1.4. The Role of Ca^{2+} Channels

It is generally accepted that the neurotransmitter release originated from vesicles is an external Ca^{2+}-dependent process, whereas efflux from the cytoplasm is not subjected to intracellular Ca^{2+} concentrations. The importance of Ca^{2+} in the regulation of somatodendritic 5-HT release was shown by the fact that omission of Ca^{2+} from the superfusion buffer completely blocked the electrical stimulation-induced [^{3}H]5-HT release whereas the resting outflow was not affected [11]. This finding also indicates that somatodendritic [^{3}H]5-HT release in the raphe nuclei could be of vesicular origin.

Since autoreceptor-mediated feedback inhibition of neurotransmitter release originates from the vesicular stores but not from the cytoplasm [169] we have speculated that Ca^{2+} availability may be a key factor for autoregulation of somatodendritic 5-HT release. The actual state of N-type somatodendritic Ca^{2+}-channels substantially regulates neurotransmitter release process: closing of voltage-sensitive Ca^{2+} channels will lead to decrease of $[Ca^{2+}]$i required for vesicular 5-HT release, whereas opening of Ca^{2+} channels may increase $[Ca^{2+}]$i which then makes exocytotic release of 5-HT possible. It was reported that 5-HT_{1A} receptor stimulation results in reduction of high threshold Ca^{2+} current in current clamp studies of acutely isolated raphe neurons [123]. Accordingly, membrane hyperpolarization that occurs after 5-HT_{1A} receptor stimulation and K^+-channel opening may be accompanied by closing voltage sensitive Ca^{2+} channel. Thus, both opening of K^+-channels and closing Ca^{2+}-channels may be involved in development of 5-HT_{1A} autoreceptor-mediated feedback inhibition of [^{3}H]5-HT release in raphe nuclei slices [78].

3.2. 5-HT_2 Receptor-Mediated 5-HT Release in the Raphe Nuclei

The involvement of 5-HT_2 receptors in the regulation of neuronal circuitry in the raphe nuclei was evidenced by recent electrophysiological experiments [101, 147]. DOI, a 5-HT_2 receptor agonist, inhibits cell firing in dorsal raphe nucleus mediated predominantly by 5-HT_{2A} receptor subtype although 5-$HT_{2B/C}$ may also be involved [36]. 5-HT_2 receptor antagonists alone had no effect on 5-HT cell firing rate, suggesting that the 5-HT_2 receptor feedback system is probably not tonically active. 5-HT_2 receptors can be activated under conditions when biophase concentration of 5-HT is elevated by selective 5-HT reuptake inhibitors. Although cellular expression of 5-HT_2 receptors in the raphe nuclei is not clear, the presence of 5-HT_2 receptor mRNA in this region was demonstrated [177]. Presynaptic interaction may also exist between 5-HT_{1A} and 5-HT_2 receptor functions in the raphe nuclei and 5-HT_2 receptor activation which facilitates presynaptic 5-HT_{1A} receptor function [94]. Moreover, after repeated administration of the 5-HT_2 receptor agonist DOI, 5-HT_2 receptors are down regulated and somatodendritic 5-HT_{1A} receptor inhibition on 5-HT neuronal firing is attenuated [94].

Others believe that 5-HT_2 receptors are probably not located presynaptically on serotonergic neurons [36, 46]. 5-HT_2 receptors may be present postsynaptically on GABA interneurons in the dorsal raphe nucleus. This conclusion was supported by the observation that 5-HT induces an increase in the frequency of inhibitory postsynaptic current of 5-HT cells by exciting GABAergic interneurons *via* 5-$HT_{2A/2C}$ receptors [101]. 5-$HT_{2A/2C}$ receptors located on GABA interneurons within the dorsal raphe nucleus mediate a local inhibitory feedback onto adjacent 5-HT neurons [101]. 5-HT_2 receptors located on cortico-raphe glutamatergic neurons may regulate GABA cell activity by trans-synaptic mechanisms in the raphe nuclei [106]. It was, however, also suggested that 5-$HT_{2A/2C}$ might be present on glutamatergic axon terminals in the raphe nuclei [12]. Moreover, it was shown that cortical 5-HT_2 receptors exert stimulation of neural circuitry of the raphe nuclei by increasing glutamate release [6].

The activation state of postsynaptic 5-HT_2 depends on biophase concentration of 5-HT. Thus, stimulation of somatodendritic 5-HT_{1A} receptors decreases extracellular concentrations of 5-HT, which may then lead to an attenuated 5-HT_2 receptor-mediated effect in the raphe nuclei. The role of postsynaptic 5-HT_2 receptors in the local regulation of raphe circuitry was suggested by our findings that the 5-HT_2 receptor agonists DOI and mCPP stimulated [^{3}H]glutamate release (Table **4**). This stimulation was however, not associated with influences of 5-HT release, suggesting that the 5-HT_2 receptor-mediated serotonergic-glutamatergic neural interactions

Table 4. Effect of 5-HT_2 Receptor Ligands on Electrical Stimulation-Induced on [^{3}H]5-HT and [^{3}H]Glutamate Release in Raphe Nuclei Slices of the Rat

Compounds	Concentration (μM)	[^{3}H]5-HT release	[^{3}H]Glutamate release
		(S2/S1)	(% of content)
Control	-	0.92±0.06	4.59±0.46
DOI	1	0.91±0.05	7.76±1.20*
mCPP	1	0.84±0.02	6.57±0.48*
Ketanserin	10	0.94±0.10	-

Raphe nuclei slices were loaded with [^{3}H]5-HT or [^{3}H]glutamate, superfused, and stimulated electrically (20 V, 2 Hz, 2-msec for 2 min) in fractions 4(S1) and 15(S2) and the release of [^{3}H]5-HT was expressed by the S2/S1 ratio. [^{3}H]Glutamate release was stimulated electrically (20 V, 20 Hz, 2-msec, for 3 min) in fraction 10 and the release was expressed as per cent of content released per stimulus. Drugs were added from fraction 7. DOI: 2,3-dimethoxy-4-iodoamphetamine, mCPP: meta-chlorophenylpiperazine. ANOVA and Dunnett's test, *P<0.05, mean±S.E.M, n=3-8.

are not part of the reciprocal innervations shown between these two neurotransmitter systems [79].

3.3. 5-HT_3 Receptor-Mediated 5-HT Release in the Raphe Nuclei: Feedback Stimulation

Although different 5-HT_1 receptor subtypes mediate autoinhibition of 5-HT release in a number of brain areas, feedback stimulation of 5-HT release by 5-HT_3 receptors has also been reported [107]. Moreover, it has been proposed that inhibitory 5-HT_{1D} autoreceptors and facilitatory 5-HT_3 autoreceptors may interact in the release-regulating process in the same serotonergic axon terminals even in raphe tissue [10, 107]. Other study concluded that 5-HT_3 receptors in the hypothalamus influence 5-HT release as heteroreceptors [30]. Besides hypothalamus, it has been shown that 5-HT_3 receptor stimulation also evokes increase in electrically induced 5-HT release from guinea-pig cerebral cortex or hippocampus, brain areas containing serotonergic axon terminals [29, 66].

3.3.1. Effects of Agonist

The 5-HT_3 receptor agonist 2-methyl-5-HT increased resting [^{3}H]5-HT release from raphe nuclei slices of the rat preloaded with [^{3}H]5-HT with an EC_{50} value of 5.3 μmol/l (Fig. **8A**). The facilitatory effect of 2-methyl-5-HT on basal [^{3}H]5-HT release was shown not only in the raphe nuclei but also in the hippocampus, brain regions that contain the central and peripheral parts of serotonergic neurons, respectively. The calculated EC_{50} value for 2-methyl-5-HT to increase [^{3}H]5-HT release from hippocampal slices was 1.15 μmol/l. 2-Methyl-5-HT was able to enhance not only the resting [^{3}H]5-HT outflow from preloaded slice preparations [29] but also the basal efflux of [^{3}H]dopamine in striatal slices [22, 145]. The latter effect was explained by reverse-mode operation of dopamine transporter.

2-Methyl-5-HT also increased the electrically evoked [^{3}H]5-HT release from rat raphe nuclei slices preloaded with

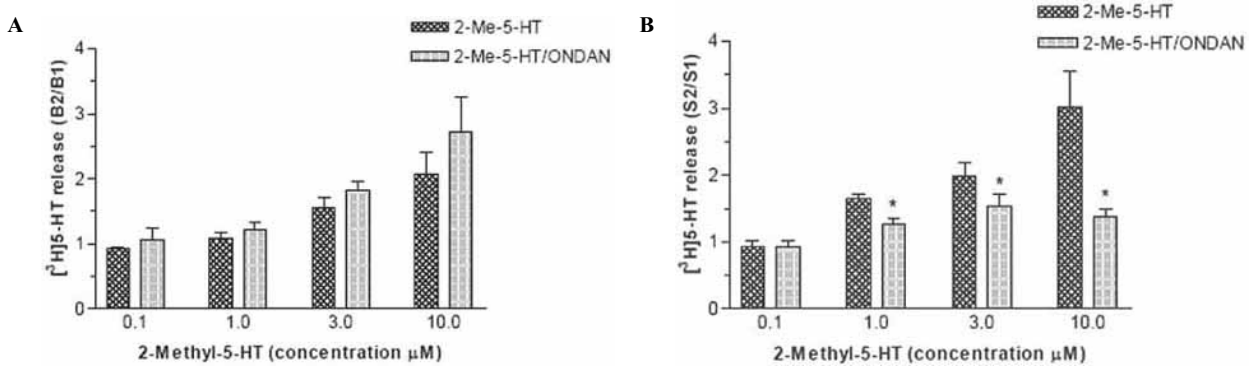

Fig. (8). The stimulatory effect of 2-methyl-5-HT (0.1 to 10 μmol/L) on (A) resting and (B) electrically stimulated [^{3}H]5-HT release from raphe nuclei slices. Slices containing the raphe nuclei were prepared from rat brain, loaded with [^{3}H]5-HT and superfused with Krebs-bicarbonate buffer. Fractional outflow of [^{3}H]5-HT was measured in fractions 3(B1) and 14(B2) and was expressed as B2/B1. The slices were stimulated electrically (40 V, 2 Hz, 2-msec for 2 min) in fractions 4(S1) and 15(S2) and the ratio of the release for S2 and S1 (S2/S1) was calculated. 2-Methyl-5-HT was added from fraction 12 and ondansetron (1 μmol/l) was added from fraction 8 and the drugs were maintained for the rest of the experiment. Ondansetron did not antagonize the stimulatory effect of 2-methyl-5-HT on resting [^{3}H]5-HT release whereas the effects of 2-methyl-5-HT on electrically stimulated [^{3}H]5-HT overflow was reversed by ondansetron. ANOVA and Dunnett's test, *P<0.05, mean±S.E.M., n=3-4.

[^{3}H]5-HT (Fig. **8B**). We found that about one magnitude lower concentration of 2-methyl-5-HT elicited an increase in depolarization-induced 5-HT release than that was required for elevation of the resting release. The calculated EC_{50} values of 2-methyl-5-HT to induce 50% increase in [^{3}H]5-HT overflow above the control were 0.56 and 0.25 μmol/l in raphe nuclei slices and hippocampal slices, respectively. Galzin and coworkers [66] found that 2-methyl-5-HT was capable of increasing depolarization -induced [^{3}H]5-HT release in guinea-pig cerebral cortex already in concentrations, which were ineffective in alteration of basal outflow. These data present evidence for the existence of 5-HT_3 receptors in the rat raphe nuclei: these receptors stimulate the release of 5-HT from the somatodendritic part of serotonergic projection neurons. The *in vitro* stimulatory effect of 2-methyl-5-HT on electrically induced 5-HT release observed in our experiments may be consonant with previous *in vivo* findings: Martin and coworkers [107] reported that 2-methyl-5-HT increases 5-HT release from hippocampus and the 5-HT_3 receptor antagonist MDL 72222 antagonized this effect.

3.3.2. Effect of Antagonists

The competitive 5-HT_3 receptor antagonist ondansetron when added alone, did not influence the electrical stimulation-induced [^{3}H]5-HT release (Table **2**). The lack of effect of ondansetron on 5-HT release indicated that there was no endogenous 5-HT tone at 5-HT_3 receptors in raphe nuclei slice preparation. The ineffectiveness of 5-HT_3 receptor antagonists on [^{3}H]5-HT release may also be explained by an extrasynaptic location of 5-HT_3 receptors and because of this location, endogenous 5-HT may reach only low concentrations at the vicinity of these receptors. This assumption was supported by increasing frequency of stimulation from 2 Hz to 10 Hz: indeed at higher frequency, ondansetron reduced [^{3}H]5-HT release in raphe nuclei slices [11].

Ondansetron was not able to antagonize the stimulatory effect of 2-methyl-5-HT on resting [^{3}H]5-HT outflow in raphe nuclei slices (Fig. **8A**). The lack of antagonistic effect of ondansetron on 2-methyl-5-HT-induced resting [^{3}H]5-HT release suggests that this effect is not a receptor-mediated event. On the contrary, ondansetron reversed the stimulatory effect of 2-methyl-5-HT on electrical stimulation-induced [^{3}H]5-HT release (Fig. **8B**) and the calculated pA_2 value for ondansetron was 6.51. The fact, that the stimulatory effect of 2-methyl-5-HT on depolarization-induced but not on spontaneous [^{3}H]5-HT release was antagonized by ondansetron, suggests the involvement of 5-HT_3 receptors in the facilitatory effect of 2-methyl-5-HT on depolarization-induced 5-HT release.

3.3.3. Mode of Action

Although 2-methyl-5-HT increased both the spontaneous and depolarization-induced release of [^{3}H]5-HT from superfused raphe nuclei slices, the mechanisms, whereby the enhanced release occurs may be different. The increased outflow of [^{3}H]5-HT in resting conditions is probably due to an exchange diffusion, whereas the increase in electrical stimulation-induced release is likely to be the result of mobilization of 5-HT stored in vesicles. This conclusion was supported by the observation that the activity of 2-methyl-5-HT in enhancing resting [^{3}H]5-HT release did not require the addition of external Ca^{2+} into the superfusion medium. Omission of Ca^{2+} from the superfusion buffer, which completely blocked the electrical stimulation-induced [^{3}H]5-HT release, failed to alter the stimulatory effect of 2-methyl-5-HT on basal [^{3}H]5-HT efflux (Table **5**).

Other experiments also suggest the involvement of 5-HT transporter in the effect of 2-methyl-5-HT on basal [^{3}H]5-HT outflow. Thus, the 5-HT uptake inhibitor fluoxetine completely blocked the stimulatory effect of 2-methyl-5-HT on basal [^{3}H]5-HT efflux (Table **5**). 2-Methyl-5-HT may be a substrate for the 5-HT transporter promoting exchange diffusion by reversal of the carrier that transports 5-HT from the extracellular space into the cytoplasmic storage sites. It is less possible that 2-methyl-5-HT acts as blocker of 5-HT transporter and increases outflow of radioactivity by preventing uptake of released 5-HT. 2-Methyl-5-HT-altered 5-HT outflow was also blocked by the 5-HT uptake blocker paroxetine in guinea-pig hypothalamic slices [29]. The effect of 2-methyl-5-HT on [^{3}H]5-HT release was further investigated in raphe nuclei slices dissected from reserpine-pretreated rats. Reserpine pretreatment, which induces redistribution of [^{3}H]5-HT from the vesicular pool to the favor of cytoplasmic

Table 5. Effect of 2-methyl-5-HT on Resting [^{3}H]5-HT Release in Raphe Nuclei Slices of Rat in Various Experimental Conditions

Conditions	Resting [^{3}H]5-HT release	
	Control group	2-Methyl-5-HT treatment
	(Percent of content per 3 min)	
Control	0.75±0.01	1.93±0.05
Ca^{2+}-free	0.99±0.17	1.57±0.27
Fluoxetine	1.25±0.13	1.10±0.08*
Reserpine	1.79±0.63*	3.07±0.49*

Raphe nuclei slices from the rat were incubated with [^{3}H]5-HT and superfused. 2-Methyl-5-HT (10 μmol/l) was added to the tissue in fraction 11 and maintained through the rest of the experiment. Basal [^{3}H]5-HT outflow was measured in fraction 3 (control group) and in fraction 14 (2-methyl-5-HT-treated group). Ca^{2+}-free buffer with 1 mmol/l EGTA and fluoxetine (1 μmol/l) were introduced at the beginning of the superfusion. Reserpine (5 mg/kg ip) was injected 18 hours before the experiments. ANOVA followed by the Dunnett's test, *P<0.05, mean±S.D., n=4.

compartments, did not alter the effect of 2-methyl-5-HT on [^{3}H]5-HT release (Table **5**).

The presented data suggest that receptor-coupled ion channels as well as voltage-sensitive Ca^{2+} channels may be involved in the 5-HT_3 receptor-mediated 5-HT release. When 5-HT_3 receptor is activated, the receptor-linked ion channel permeable to Na^+, K^+ and Ca^{2+} opens up and Na^+ influx will lead to local membrane depolarization [52, 178]. The consequent increase in [Na^+]i concentrations will result in operation of 5-HT transporters in reverse mode and 5-HT outflow from the cytoplasmic pool to the synaptic cleft will occur. In addition, membrane depolarization evokes opening of voltage-sensitive Ca^{2+} channels and the elevated Ca^{2+} influx will lead to exocytotic release of 5-HT from the vesicular pool. In fact, we have found that 2-methyl-5-HT increases both resting and electrical stimulation-induced [^{3}H]5-HT release from raphe nuclei slices, releases that originate from cytoplasmic and vesicular transmitter pools, respectively [11].

3.4. 5-HT_7 Receptor-Mediated 5-HT Release in Raphe Nuclei

5-HT_7 receptors are novelly discovered member of 5-HT receptors [164], which is positively coupled to adenylyl cyclase through Gs protein [14]. Although no selective agonist for 5-HT_7 receptors is yet available, 5-carboxamidotryptamine (5-CT) and 5-methoxytryptamine express high affinity and 8-OH-DPAT binds with moderate affinity to 5-HT_7 receptors [14, 86, 135]. SB-258719, SB-258741, and SB-269970 were the first selective 5-HT_7 receptor antagonists [61, 69, 102], which were recently introduced as research tools. The identification of 5-HT_7 receptor mRNA in the raphe nuclei and of 5-HT_7 receptor protein in serotonergic cell bodies and axon terminal fields raised the possibility that these receptors operate as 5-HT release-mediating autoreceptors [67, 135, 161]. 5-HT_7 receptors, however, may exert inhibition on [^{3}H]5-HT release indirectly in raphe nuclei serving as heteroreceptors rather than autoreceptors in release inhibition and in fact, observations that exclude the autoreceptor function of 5-HT_7 receptors have also been reported [132].

3.4.1. Effect of Agonist

The non-selective 5-HT receptor agonist 5-CT did not influence the resting [^{3}H]5-HT release from raphe nuclei slices of the rat. However, 5-CT inhibited the electrically evoked [^{3}H]5-HT release in a concentration-dependent manner (Fig. **9**). 5-CT has nanomolar affinity for 5-HT_7 receptors [33, 85], it also binds to 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors although with lower affinities [32, 72, 171]. In addition, 5-CT also expresses affinity for 5-HT_5 receptors [57] whereas it is less potent at 5-HT_4 receptors [38]. Boess and Martin [33] have reported that the affinity of 5-CT for 5-HT_4 receptors is approximatively 200-fold lower than for 5-HT_7 receptors. These findings suggest that 5-CT inhibits [^{3}H] 5-HT release in slices of the rat raphe nuclei and multiple 5-HT receptors may be involved in this effect. Besides 5-CT, the highly specific 5-HT_{1A} receptor agonist 8-OH-DPAT [109] also has affinity for the 5-HT_7 receptors although its agonist potency is less with about two magnitudes than that of 5-CT [85, 160]. We have shown previously that the estimated IC_{50} value of 8-OH-DPAT to inhibit [^{3}H]5-HT release was 280 nM (Fig. **5**).

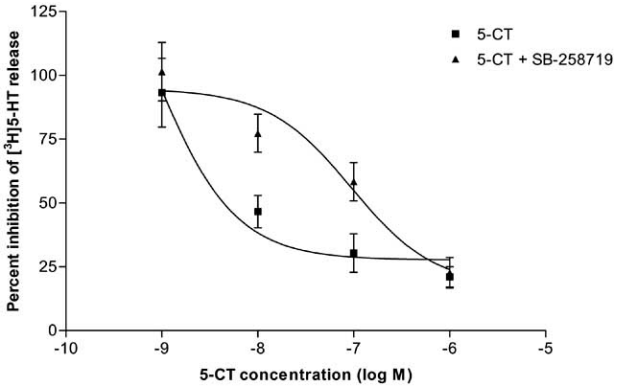

Fig. (9). Effect of SB-258719 (10 μM) on 5-CT-induced inhibition of [^{3}H]5-HT release in raphe nuclei slices of the rat. The IC_{50} value of 5-CT to inhibit [^{3}H]5-HT release was 3.34±0.37 nM and it was 94.23±4.84 nM in the presence of 10 μM SB-258719. 5-CT was added 9 min and the 5-HT_7 receptor antagonist was added 18 min before S2. Mean±S.E.M., n=3-4.

3.4.2. Effect of Antagonist

The selective 5-HT_7 receptor antagonist SB-258719 [61] did not influence electrical stimulation-induced [^{3}H]5-HT release from raphe nuclei slices and the 5-HT_6 receptor antagonist SB-271046 also failed to modify the transmitter outflow (Table **2**). Furthermore, SB-258719 did not stimulate basal or electrical stimulation-induced [^{3}H]5-HT release on its own but produced an antagonism of 5-CT response (Fig. **9**). SB-258719 produced a concentration-dependent rightward shift of the concentration-[^{3}H]5-HT release inhibition curve of 5-CT with no significant alteration in the maximal 5-CT response. Schild analysis for SB-258719 gave a pA_2 of 6.43. This value is lower than the pK_B and pKi values of SB-258719 in 5-CT-stimulated adenylate cyclase assay or in [^{3}H]5-CT binding experiments [160]. Although the reason of this discrepancy is not clear it should bear in mind the complexity of brain slices as compared with cells or membrane preparations as well as the physico-chemical characteristics of compounds which determines diffusion of a compound into brain slices.

3.4.3. Mode of Action

[^{3}H]5-CT does not selectively label 5-HT_7 receptors in native tissues and it has therefore been necessary to include selective blocking compounds to prevent labeling of non-5-HT_7 binding sites [39, 156, 161, 172]. We have measured raphe [^{3}H]5-HT release in the presence of various 5-HT_1 receptor antagonists in order to prevent stimulation of non-5-HT_7 receptors by 5-CT. The combination of WAY-100635 with SB-216641 resulted in an additive inhibition in [^{3}H]5-HT release further pointing out the role of 5-HT_1 receptor subtypes in mediation of 5-CT-induced 5-HT release inhibition [80]. In addition, SB-258719 induced a further rightward shift of the [^{3}H]5-HT release inhibition curve of 5-CT determined in the presence of SB-216641. The fact, that SB-216641 and SB-258719 both antagonized the 5-CT-induced [^{3}H]5-HT release inhibition and their effects were additive in nature, indicates that 5-$HT_{1B/1D}$ and 5-HT_7 receptors are

probably independently involved in the regulation of the 5-HT release in the raphe nuclei. These findings confirm that more than one types of 5-HT receptors participate in the 5-CT-induced inhibition of [^{3}H]5-HT release, i.e. 5-HT$_7$ as well as 5-HT$_1$ receptor subtypes possess regulatory role in the release process. It is possible that various 5-HT receptors synergistically act to finely control 5-HT release: 5-HT$_{1B/1D}$ receptors may be under tonic influence of 5-HT released, whereas 5-HT$_{1A}$ and 5-HT$_7$ receptors may influence 5-HT release with a more phasic mode. 5-HT receptors involved in regulation of 5-HT release are located differently as 5-HT$_{1B/1D}$ receptors may be expressed on recurrent axon collaterals or axon terminals and 5-HT$_{1A}$ receptors are probably located in the somatodendritic area. Since 5-HT is concentrated into different neuronal pools it is possible that 5-HT$_7$ and 5-HT$_1$ receptor subtypes regulate 5-HT release originated from different stores of the same neurons or from different neurons. Experiments carried out with tetrodotoxin (TTX) suggest that 5-HT$_7$ receptors may not serve as autoreceptors but rather have heteroreceptor functions in the raphe nuclei.

4. HETERORECEPTOR-MEDIATED NEUROTRANSMITTER RELEASE IN THE RAPHE NUCLEI

4.1. Reciprocal Innervation Between Serotonergic and Glutamatergic Neurons

To determine whether 5-HT$_7$ receptors serve as auto- or heteroreceptors in the regulation of 5-HT release in the raphe nuclei, the effect of 5-CT on [^{3}H]5-HT release was measured in the presence and absence of the voltage-sensitive Na^+-channel blocker, TTX. Application of TTX has importance to locate receptors regulating neurotransmitter release and in our experiments, to characterize auto- or heteroreceptor function of 5-HT receptors involved in 5-HT release. TTX inhibits electrically evoked [^{3}H]5-HT release presumably by blocking conduction in the electrically excitable membrane [152]) and thus by inhibition of action potential propagation. On the contrary, TTX does not block [^{3}H]5-HT release evoked by elevated KCl, a depolarizing stimulus that appears to act directly on axon terminals or dendritic buttons [76]. Therefore, elevated KCl instead of electrical stimulation was used to elicit [^{3}H]5-HT overflow when TTX was applied.

5-CT inhibited the KCl-induced [^{3}H]5-HT release similarly to those experiments when electrical stimulation was used to evoke [^{3}H]5-HT release (Fig. **10**). Addition of TTX prevented the inhibitory effect of 5-CT on K^+-stimulated [^{3}H]5-HT release from raphe nuclei slices suggesting that 5-HT$_7$ receptors may not serve as autoreceptors but act as heteroreceptors in controlling 5-HT release. We speculated that the 5-HT$_7$ receptor-mediated 5-HT release inhibition may involve operation of another, possibly excitatory neurotransmitter system. These postulated excitatory neurons may be glutamatergic and therefore, the regulatory role of 5-HT$_7$ receptors in glutamate release was further studied in the raphe nuclei slice preparation.

4.1.1. 5-HT$_7$ Receptor-Mediated Glutamate Release in the Raphe Nuclei

We found that 5-CT inhibited [^{3}H]glutamate release from raphe nuclei slices and this inhibition was reversed by the selective 5-HT$_7$ receptor antagonist SB-258719 (Fig. **11**). This finding suggests that glutamatergic axon terminals in the raphe nuclei express 5-HT$_7$ receptors and their stimulation leads to inhibition of glutamatergic excitatory tone. The activation state of 5-HT$_7$ heteroreceptors located on glutamatergic axon terminals may depend on biophase concentration of 5-HT and increase in extracellular 5-HT concentration

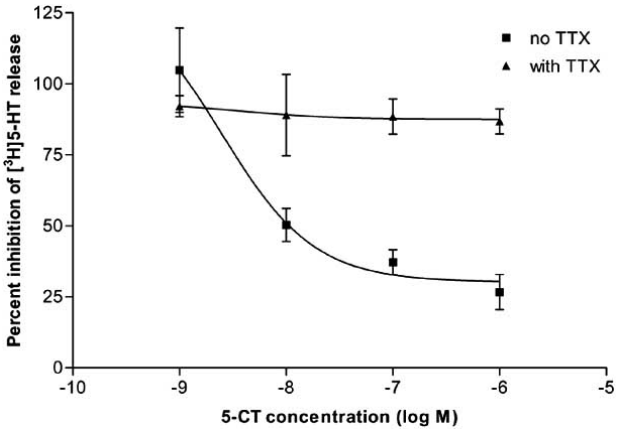

Fig. (10). Effect of TTX on 5-CT-induced inhibition of [^{3}H]5-HT release in raphe nuclei slices of the rat. Slices containing the raphe nuclei were prepared from rat brain, loaded with [^{3}H]5-HT and superfused with Krebs-bicarbonate buffer in the presence and absence of TTX (1 μM). The slices were stimulated by elevated KCl (22 mM for 2 min) in fractions 4(S1) and 15(S2). 5-CT (0.001 to 1 μM) was added 9 min before electrical stimulation and was maintained throughout the experiment. In order to block 5-HT$_1$ receptor subtypes, WAY-100635 (10 μM) and SB-216641 (1 μM) were added to the superfusion buffer. KCl-induced [^{3}H]5-HT release expressed as S2/S1 that was taken as 100% was 0.76±0.03 in the absence and it was 0.93±0.12 in the presence of TTX (P>0.05, n=3-4). Mean±S.E.M., n=3-4. (Figure taken from Reference [80]).

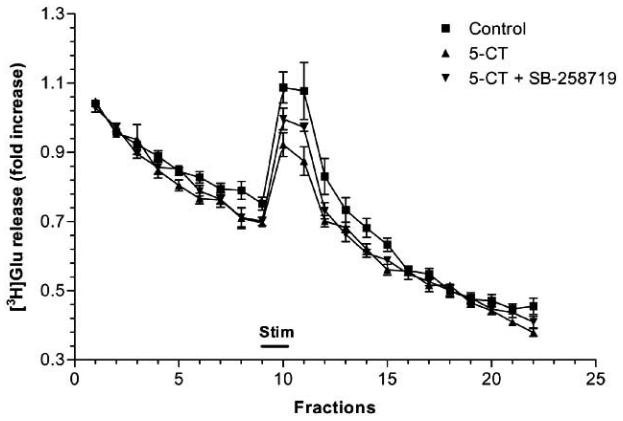

Fig. (11). The effects of 5-CT and 5-CT and SB-258719 on [^{3}H]glutamate release from raphe nuclei slices of the rat. The slices were stimulated electrically (20 V, 2 Hz, 2-msec for 2 min) in fraction 10. 5-CT (0.1 μM) was added 9 min and SB-258719 (10 μM) was added 18 min before electrical stimulation of [^{3}H]glutamate release. In order to block 5-HT$_1$ receptor subtypes, WAY-100635 (10 μM) and SB-216641 (1 μM) were added to the superfusion buffer. Mean±S.E.M., n=3-4.

leads to inhibition of 5-HT_7 receptor-mediated glutamate release in the raphe nuclei.

4.1.2. Glutamate Receptor-Mediated 5-HT Release in the Raphe Nuclei

Whether the postulated serotonergic-glutamatergic interaction is reciprocal in nature, the influence of glutamate receptor agonists on [^{3}H]5-HT release was also determined. As shown in Fig. (**12A**), the ionotropic glutamate receptor agonists NMDA and AMPA evoked increased [^{3}H]5-HT release from raphe nuclei slices of the rat. In another series of experiments, the NMDA receptor antagonist MK-801 antagonized the inhibitory effect of 5-CT on electrically stimulated [^{3}H]5-HT release from raphe nuclei slices of the rat [80] further indicating that 5-HT_7 receptors indirectly, *via* glutamate release, influence serotonergic neural activity.

4.1.3. Serotonergic-Glutamatergic Circuitry in the Raphe Nuclei

5-HT_7 receptor immunoreactivity in neural fibers and cell bodies of the raphe nuclei was demonstrated by using antibody produced against 5-HT_7 receptors [53]. These receptors are probably not located on 5-HT-containing neurons within the raphe nuclei as was suggested by our experiments employing the neurotoxin TTX. The functional and morphological observations presented above raise the possibility that 5-HT_7 receptors do not serve as autoreceptors but they act as heteroreceptors in controlling 5-HT release in the raphe nuclei. A glutamatergic pathway rises from the median prefrontal cortex and projects to the raphe nuclei [71] and the primary target for the cortico-raphe glutamatergic neurons is the raphe 5-HT neurons [159]. There might be a local interaction between glutamatergic axon terminals and serotonerg projection neurons, which may represent a fine tune between the main neural cell types in the raphe nuclei (Fig. **13**). Increase in extracellular 5-HT concentration leads to inhibition of 5-HT_7 receptor-mediated glutamate release which then may reduce excitatory input to serotonerg neurons and the further release of 5-HT will be attenuated. The serotonergic-glutamatergic interaction may be reciprocal in nature as the glutamatergic agonists NMDA and AMPA evoked increase in 5-HT release and this finding confirms previously presented data [159]. The local glutamatergic and serotonergic circuitry postulated in the raphe nuclei forms an excitatory-inhibitory connection by which incoming excitatory signals are converted into inhibitory output projecting to various brain areas like cerebral cortex, striatum, hippocampus or hypothalamus. This circuitry may also involve GABAergic interneurons as was suggested by the findings that NMDA and AMPA also evoke GABA release from raphe nuclei slices (Fig. **12B**).

4.2. Reciprocal Innervation Between Serotonergic and GABAergic Neurons

A great number of experiments indicate that serotonergic projection neurons and GABA interneurons present in the raphe nuclei mutually regulate each other's activity [62, 64]. Thus, GABA inhibits serotonergic systems as it decreases the turnover rate of 5-HT in the raphe nuclei and it has also been reported that serotonergic neural activity was blocked by local application of GABA agonists in the raphe nuclei [58]. Besides the autoreceptor characteristics of 5-HT_1 receptor subtypes, these receptors may also serve as heteroreceptors expressed on GABA interneurons and serotonergic neurons also possess GABA heteroreceptors, which are involved in mediation of GABAergic inhibition [158]. To asses reciprocal pattern of serotonergic-GABAergic interactions in the raphe nuclei, slices of rat raphe nuclei were loaded with either [^{3}H]5-HT or [^{3}H]GABA in order to measure radiolabeled neurotransmitter release and the role of 5-HT_1 and GABA receptor subtypes in this regulation was determined. Using this approach, we observed that (1) 5-HT inhibits [^{3}H]GABA release by 5-HT_{1A} and 5-HT_{1B} receptors and (2) GABA inhibits [^{3}H]5-HT release by $GABA_A$ and $GABA_B$ receptors located on 5-HTergic neurons [12]. An effort has been made to elucidate the location of receptors involved in this reciprocal innervation.

4.2.1. Serotonergic Modulation of GABA Release in the Raphe Nuclei

As it is shown in Table **6**, the 5-HT_{1A} receptor agonist 8-OH-DPAT and the 5-HT_{1B} receptor agonist CGS-12066 de

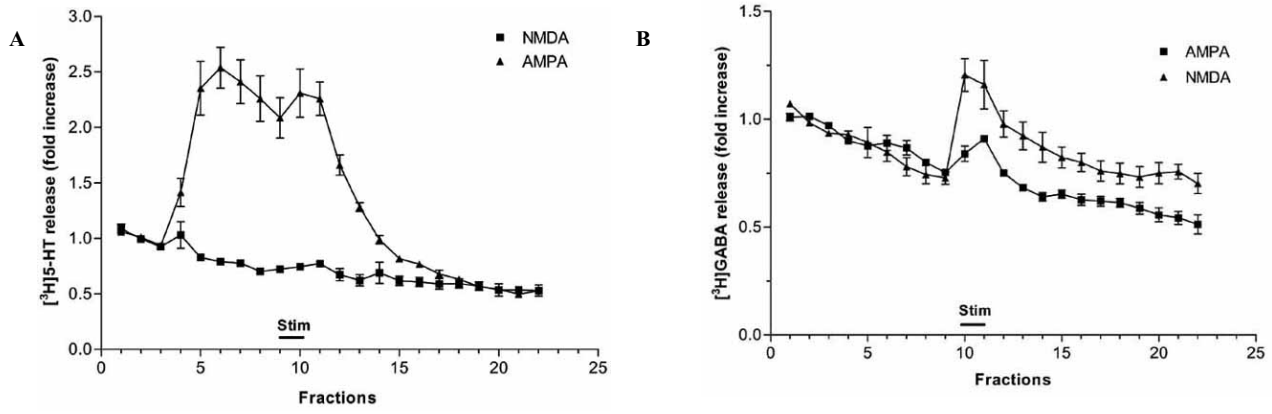

Fig. (12). Effects of NMDA and AMPA on (A) [^{3}H]5-HT and (B) [^{3}H]GABA release from raphe nuclei slices of the rat. The slices were superfused with NMDA (0.3 mM) or AMPA (0.3 mM) in fractions 10 and 11. When AMPA was used, cyclothiazide was also added in a concentration of 0.3 mM from fraction 4. Mean±S.E.M., n=3-4.

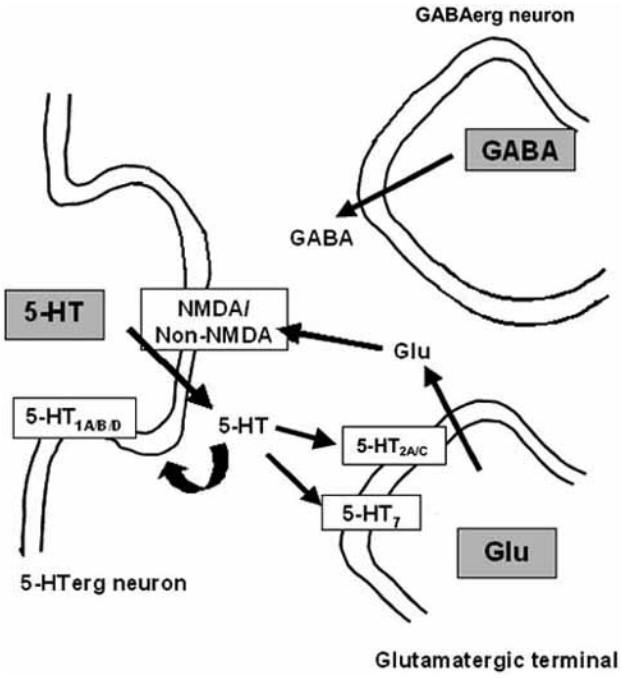

Fig. (13). A model for interaction between serotonergic and glutamatergic neurons in the raphe nuclei of the rat. 5-HT released from dendrites or recurrent axon collaterals of serotonergic neurons inhibits its own release by release-mediating 5-HT_{1A} and 5-$HT_{1B/1D}$ receptors. Furthermore, 5-HT released into the biophase may inhibit glutamate release either in a synaptic or non-synaptic communication by 5-HT_7 heteroreceptors located on glutamatergic axon terminals. Activation of 5-HT_7 receptors on glutamatergic neurons by 5-HT inhibits glutamate release and reduced excitatory influence will lead to inhibition of 5-HT release. The indirect regulation of 5-HT release by 5-HT_7 heteroreceptors was evidenced by the fact that TTX suspended the inhibitory effect of 5-CT on 5-HT release. Glutamatergic axon terminals in the raphe nuclei may also possess excitatory 5-$HT2_{A/2C}$ receptors as was suggested by Bagdy *et al*., [12]. Raphe nuclei obtain glutamatergic innervation via the cortico-raphe projection neurons originated from the medial prefrontal cortex [71] and the latter receives inhibitory influences from the raphe-cortical serotonergic projection [116]. A possible location of 5-HT_7 receptors on raphe nuclei GABAergic neurons was suggested Roberts and coworkers [132]. We found, however, that bicuculline, a $GABA_A$ receptor antagonist, did not alter 5-CT-mediated inhibition of 5-HT release [79]. The scheme does not show astrocytes, which surround neurons and are involved in neurotransmitter efflux and uptake processes.

Table 6. Effects of 5-HT and GABA Receptor Ligands on Electrical Stimulation-Induced [^{3}H]5-HT and [^{3}H]GABA Release from Raphe Nuclei Slices of the Rat

Compounds	Receptor	Concentration (μM)	[^{3}H]5-HT release (S2/S1)	[^{3}H]GABA release (S2/S1)
Control		-	0.95±0.03	0.92±0.05
8-OH-DPAT	5-HT_{1A}	1	0.44±0.04*	0.29±0.05*
CGS-12066	5-HT_{1B}	1	0.45±0.02*	0.36±0.04*
Control		-	0.99±0.06	0.89±0.05
Muscimol	$GABA_A$	30	0.56±0.05*	0.53±0.07*
Baclofen	$GABA_B$	100	0.27±0.04*	0.31±0.09*
Control		-	0.99±0.05	0.91±0.07
(+)WAY-100135	5-HT_{1A}	1	1.08±0.04	0.94±0.13
Bicuculline	$GABA_A$	100	0.98±0.08	1.13±0.12
Phaclofen	$GABA_B$	100	2.11±0.63*	0.77±0.20

Rat raphe nuclei slices were prepared, loaded with [^{3}H]5-HT or [^{3}H]GABA, superfused and stimulated electrically in fractions 4 and 15 for determination of [^{3}H]5-HT or [^{3}H]GABA release. ANOVA followed by the Dunnett's test, *P<0.05, mean±S.E.M., n=3-7.

creased electrical stimulation-induced [^{3}H]GABA release from isolated raphe nuclei slices of the rat. The 5-HT_{1A} receptor antagonist (+)WAY-100135 did not affect [^{3}H]GABA release on its own right but antagonized the inhibitory effects of 8-OH-DPAT on electrical stimulation-evoked [^{3}H]GABA efflux (Table **6**). These findings indicate that 5-HT release originated from dendrites and/or axon terminals inhibits GABA neurons in the raphe nuclei and both 5-HT_{1A} and 5-HT_{1B} receptors are involved in this inhibition. Data presented in Table **6** also summarize the inhibitory effects of 8-OH-DPAT and CGS-12066 on [^{3}H]5-HT release in raphe nuclei.

To determine whether 5-HT_{1A} and 5-HT_{1B} receptors influencing [^{3}H]GABA release are located on GABA neurons or they are situated on other neuronal elements, the voltage-sensitive Na^+-channel blocker TTX was used. We found that TTX did not block [^{3}H]GABA overflow in response to elevated KCl, a depolarizing stimulus that appears to act directly on axon terminals [152]). Moreover, 8-OH-DPAT decreased K^+-induced [^{3}H]GABA release and this inhibition was completely abolished by TTX [12]. Although CGS-12066 also inhibited [^{3}H]GABA efflux elicited by KCl depolarization, this inhibition persisted even in the presence of TTX. Experiments carried out in slices treated with TTX shows that the location of 5-HT_{1A} and 5-HT_{1B} receptors involved in GABA release inhibition may be different and this was further evidenced in experiments carried out in 5-HT-deficient raphe slices.

5-HT stores in raphe nuclei slices were depleted by repeated administration of para-chlorophenylalanine (pCPA) to rats and the effects of 5-HT_1 receptor agonists on [^{3}H]GABA release were measured [12]. We found that in 5-HT deficient slices, CGS-12066 decreased the electrically evoked [^{3}H]GABA efflux whereas 8-OH-DPAT did not exert any inhibition on transmitter overflow (Table **7**). Thus, the inhibitory effect of 5-HT_{1B} receptors on GABA release was independent from the tissue concentration of endogenous 5-HT whereas that of 5-HT_{1A} receptors was suspended after emptying 5-HT stores. This set of experiments suggests that 5-HT_{1B} receptors are located on GABAergic axon terminals and mediate a direct inhibition of GABA release whereas 5-HT_{1A} receptors are located on 5-HT cells and they exert an indirect inhibitory effect on GABA release.

4.2.2. GABAergic Modulation of 5-HT Release in the Raphe Nuclei

As it is shown in Table **6**, the $GABA_A$ receptor agonist muscimol and the $GABA_B$ receptor agonist baclofen inhibited the electrically induced [^{3}H]5-HT overflow in the raphe nuclei slices. The $GABA_A$ receptor antagonist bicuculline did not affect the release of [^{3}H]5-HT on its own right, however, it antagonized the inhibitory effects of muscimol on electrical stimulation-evoked [^{3}H]5-HT release. The $GABA_B$ receptor antagonist phaclofen increased [^{3}H]5-HT overflow elicited by electrical stimulation and reversed the baclofen-induced inhibition of [^{3}H]5-HT efflux (Table **6**). Data presented in Table **6** also summarize the inhibitory effects of muscimol and baclofen on [^{3}H]GABA release in raphe nuclei.

Our experiments indicate that both $GABA_A$ and $GABA_B$ receptors may be involved in the GABAergic-5-HTergic neural interaction in the raphe nuclei. The dual expression of $GABA_A$ and $GABA_B$ receptors on postsynaptic membranes has been demonstrated and even interactions at the level of GABA receptor subtypes were suggested [98]. The two GABA receptor subtypes may exert different types of inhibition on serotonergic neuronal activity as the $GABA_B$ receptor antagonist phaclofen, on its own right, increased and the $GABA_A$ receptor antagonist bicuculline did not affect soma-

Table 7. Effects of 5-HT and GABA Receptor Ligands on Electrical Stimulation-Induced [^{3}H]5-HT and [^{3}H]GABA Release from Para-Chlorophenylalanine (p-CPA)- or Isoniazid-Treated Rat Raphe Nuclei Slices

Compound	Concentration (μM)	[^{3}H]5-HT	[^{3}H]GABA
		release (S2/S1)	
		pCPA-treated slices	
Control	-		0.83±0.07
8-OH-DPAT	1		0.95±0.17
CGS-12066A	1		0.49±0.06*
		Isoniazid-treated slides	
Control	-	0.93±0.02	
Muscimol	10	0.44±0.07*	
Baclofen	100	0.36±0.06*	

Slices containing raphe nuclei were prepared from rats pretreated with either pCPA or isoniazid. Rat raphe nuclei slices were loaded with [^{3}H]5-HT or [^{3}H]GABA, superfused and stimulated electrically twice for determination of [^{3}H]5-HT or [^{3}H]GABA release. Agonists were added 8 min before 2nd stimulation. ANOVA followed by the Dunnett's test, *P<0.05, mean±S.E.M., n=4-7. p-Chlorophenylalanine (pCPA), an inhibitor of tryptophan hydroxylase was injected in a dose of 100 mg/kg/day ip for 5 days. pCPA-pretreatment reduced 5-HT content in the raphe nuclei from 1.67±0.10 to 0.49±0.04 μg/g (P<0.05, n=4). Isoniazid, an inhibitor of glutamic acid decarboxylase, was injected in a dose of 450 mg/kg sc, 45 min before decapitation. Isoniazid-pretreatment reduced GABA content in the raphe nuclei from 334±13 to 189±23 μg/g (P<0.05, n=3-4). The 5-HT and GABA concentrations in the raphe nuclei slices of the rat were measured by HPLC/electrochemistry according to Mefford [108] and Rowley *et al*. [134], respectively.

todendritic 5-HT release. Tonic regulation of $GABA_B$ receptors by the endogenously released GABA suggests a possible synaptic location of these receptors whereas a more phasic regulation by $GABA_A$ receptors is in favor of non-synaptic influence on serotonergic neurons.

In a further attempt to localize $GABA_A$ and $GABA_B$ receptors regulating 5-HT release, raphe nuclei slices were taken from rats pretreated with the glutamic acid decarboxylase inhibitor isoniazid [12]. In GABA-deficient slices, muscimol and baclofen decreased the electrically evoked [^{3}H]5-HT efflux (Table **7**). This finding indicates that 5-HT release-regulating $GABA_A$ and $GABA_B$ receptors may be situated postsynaptically on 5-HT neurons since depletion of releasable GABA by isoniazid failed to alter their inhibitory effects.

4.2.3. Serotonergic-GABAergic Circuitry in the Raphe Nuclei

Local interaction between 5-HTergic projection neurons and GABAergic interneurons operates in a reciprocal fashion establishing a fine tune between the main neural cells types in the raphe nuclei (Fig. **14**). GABA exerts trans-synaptic control on 5-HT neurons as was shown by a series of biochemical, electrophysiological and morphological experiments whereas few 5-HT nerve endings were found only in contact with GABA elements [79, 114, 141]. Thus, both synaptic and non-synaptic release of neurotransmitters might be involved in the local serotonergic, GABAergic and glutamatergic circuitry of the raphe nuclei. This neural network forms complicated excitatory-inhibitory connections by which incoming excitatory signals from the cerebral cortex are converted into inhibitory output and sent back to cerebral cortical areas. In fact, 5-HT innervation of the medial prefrontal cortex originates from the raphe nuclei and the raphe-cortical 5-HT neurons mediate an inhibition in the cortical neural network [116, 154].

4.3. Interactions Between Serotonergic and Noradrenergic Neurons

Several morphological and functional investigations indicate the occurrence of interaction between serotonergic and noradrenergic systems in the midbrain dorsal and median raphe nuclei. Early histological studies demonstrated noradrenergic afferent connections projecting to the raphe nuclei [138]. The dense noradrenergic innervation arises from the lower brainstem noradrenergic cell groups: retrograde tracing method labeled neurons in the locus coeruleus (A6 noradrenergic cell group), the A5 noradrenergic cell group, the cau-

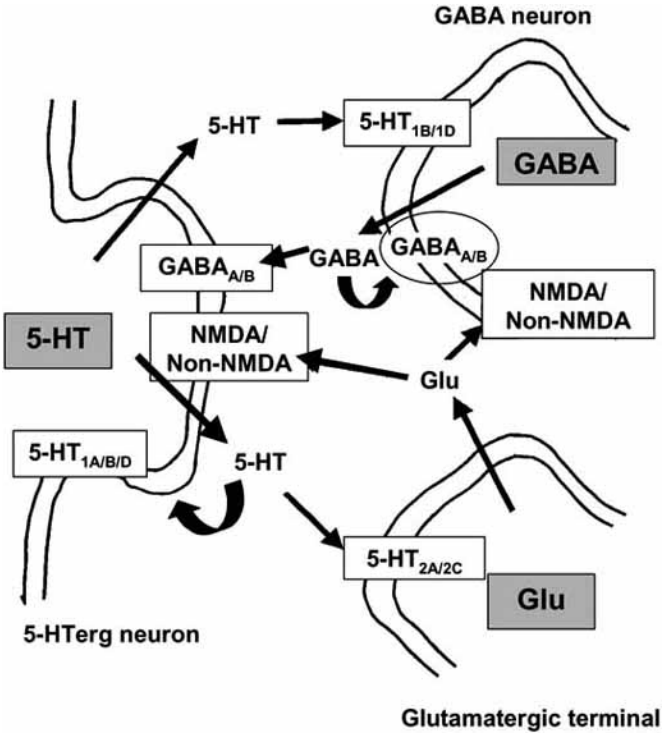

Fig. (14). A model for possible reciprocal interaction between serotonergic and GABAergic neurons in the raphe nuclei. 5-HT released from dendrites or recurrent axon collaterals of serotonergic neurons inhibits its own release by $5\text{-}HT_{1A}$ and $5\text{-}HT_{1B/1D}$ receptors. GABA-containing cells in the raphe nuclei are interneurons but these brain nuclei also receive inhibitory GABAergic projections. GABA once released inhibits its further-release through $GABA_A$ and $GABA_B$ autoreceptors located on GABAergic neurons. Moreover, GABA inhibits 5-HT release by stimulating $GABA_A$ and $GABA_B$ receptors located on 5-HTergic projection neurons. $GABA_B$ receptors may mediate a tonic inhibition whereas $GABA_A$ receptors exert a more phasic inhibition on 5-HT release. On the other hand, 5-HT inhibits GABA release by $5\text{-}HT_{1B/D}$ heteroreceptors located on GABAergic axon terminals. The $5\text{-}HT_{1A}$ autoreceptor-mediated inhibition of GABA release is probably an indirect effect and it may be mediated by excitatory $5\text{-}HT_2$ receptors located on glutamatergic axon terminals. Glutamatergic axon terminals in the raphe nuclei possess excitatory $5\text{-}HT_{2A/2C}$ receptors as was suggested by Bagdy and coworkers [12].

doventrolateral medulla and the nucleus of the solitary tract (A1 and A2 noradrenegic cell groups) [124].

Neurochemical experiments showed the presence of norepinephrine (NE) and the enzymes involved in its synthesis within the raphe nuclei [137]. Autoradiographic studies have revealed the presence of alpha-1, alpha-2 and beta adrenoceptors in the dorsal raphe nucleus [148] and the presence of alpha-1 mRNA has also been demonstrated [50, 157]. The high level of mRNA encoding the alpha-1B receptors makes it likely that this adrenoceptor subtype is responsible for noradrenergic influence on serotonergic neurons. Alpha-1 heteroreceptors mediating noradrenergic control on serotonergic neurons may be located at the cell body levels.

Alpha-2 adrenoceptor binding has also been demonstrated in the raphe nuclei [50, 157]. Immuno-histochemical studies revealed the presence of alpha-2A and alpha-2C adrenoceptor immunoreactivities in the dorsal raphe nucleus [157]. Functional tests indicated that alpha-2A adrenoceptor subtype is involved in regulation of 5-HT release [133]. This was further supported by the finding that infusion of the alpha-2A adrenoceptor antagonist BRL 44408 into the dorsal raphe nucleus caused an increase of 5-HT release [129]. The majority of alpha-2 adrenoceptors may be located in noradrenergic axon terminals where they serve as autoreceptors and they regulate primarily NE release [37]. Others pointed out that alpha-2 adrenoceptors are involved in both NE and 5-HT release in rat dorsal raphe nucleus slices [84]. Guyenet and coworkers [68] concluded, however, that only a low percentage of 5-HT cells exhibit detectable alpha-2A adrenoceptor immunoreactivity in the dorsal raphe nucleus.

4.3.1. Noradrenergic Modulation of 5-HT Release in the Raphe Nuclei

The alpha-2 adrenoceptor agonist clonidine inhibited the electrical stimulation-induced [^{3}H]5-HT release from isolated raphe nuclei slices of the rat (Fig. **15A**). This effect was reversed by addition of idazoxan, an antagonist of alpha-2 adrenoceptors (pA_2 value 6.88). Idazoxan (0.001 to 1 μM) by itself failed to influence [^{3}H]5-HT release in these experimental conditions. The present data suggest that NE released from noradrenergic axon varicosities influences somatodendritic 5-HT release in the raphe nuclei and this effect may be either a direct or an indirect inhibition mediated by presynaptic and/or postsynaptic alpha-2 adrenoceptors.

In vivo microdialysis studies also concluded that clonidine reduced and idazoxan or 2-methoxy-idazoxan enhanced 5-HT release in the rat raphe nuclei [3, 37]. These effects of alpha-2 ligands might be, however, indirect. Thus, idazoxan may enhance noradrenergic tone on 5-HT neurons through removal of the negative feedback inhibition of NE release, which then leads to activation of postsynaptic alpha-1 adrenoceptors located on 5-HT neurons. Supporting this view, it was found that the noradrenergic neurotoxin DSP-4 prevented the inhibitory effect of clonidine on 5-HT release suggesting an indirect effect on 5-HT output [37]. In raphe nuclei slice preparation, however, prazosin failed to suspend the 5-HT release-inhibitory effect of dexmedetomidine suggesting that alpha-2A heteroceptors may play a role in noradrenergic-serotonergic interaction [84].

The alpha-1 adrenoceptor antagonist prazosin (0.1 to 1 μM) had neither stimulatory nor inhibitory effect on [^{3}H]5-HT release from raphe nuclei slices. The alpha-1 adrenoceptor agonist phenylephrine inhibited electrical stimulation-induced [^{3}H]5-HT release (Fig. **15A**) and this inhibition was associated by an enhanced basal 5-HT outflow. This effect of phenylephrine is surprising as this drug is widely used to increase single neuron firing rate [89]. The opposing effect of phenylephrine on 5-HT release may be a consequence of its dual electrophysiological effect: phenylephrine evokes depolarization and increases duration of after-hyperpolarization following action potential [120]. In another series of experiments, bath addition of phenylephrine also reduced 5-HT release assessed by voltammetry in raphe slices [84].

Although *in vitro* experiments provided some evidence for the inhibitory properties of alpha-1 adrenoceptors on somatodendritic 5-HT release, *in vivo* investigations led to opposite conclusion. Using microdialysis technique in freely

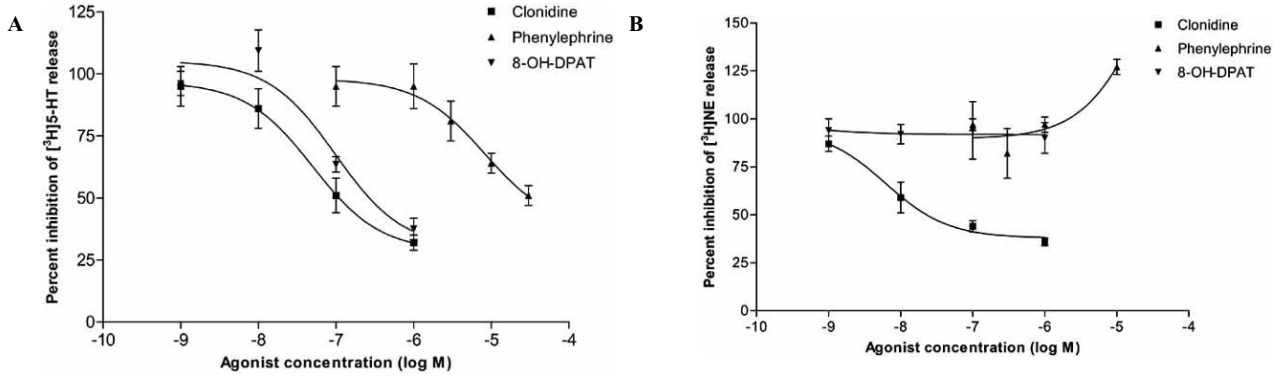

Fig. (15). Effects of clonidine, phenylephrine and 8-OH-DPAT on [^{3}H]5-HT (A) and [^{3}H]NE (B) release from raphe nuclei slices of the rat. The calculated IC_{50} values to inhibit [^{3}H]5-HT release were 50.26±9.27 and 98.62±3.16 nM for clonidine and 8-OH-DPAT, and it was 8.31±0.67 μM for phenylephrine. Clonidine inhibited [^{3}H]NE release with an IC_{50} value of 6.18±0.64 nM, phenylephrine and 8-OH-DPAT were ineffective. For experimental procedure see Fig. (**2**). Drugs were added to the tissue from fraction 10 and maintained through the experiment. Mean±S.E.M., n=3-4.

moving rats, phenylephrine did not modify 5-HT output in the median raphe nucleus, whereas prazosin reduced the release of 5-HT [3]. This finding suggests that endogenous NE exerts a direct tonic stimulatory control on 5-HT release through alpha-1 adrenoceptors and these receptors may be maximally stimulated in resting conditions [129].

4.3.2. Noradrenergic Modulation of Norepinephrine Release in the Raphe Nuclei

Clonidine, added to raphe nuclei slices in concentrations of 0.001 to 1 μM, also inhibited the electrical stimulation-induced [^{3}H]NE release (Fig. **15B**) (pA_2 value 7.75). This finding can sufficiently be explained by an effect of clonidine on release-mediating alpha-2 presynaptic autoreceptors located on nora-drenergic nerve terminals. Idazoxan reversed the clonidine-induced NE release inhibition. In contrast to [^{3}H]5-HT release, [^{3}H]NE release was concentration-dependently increased by addition of idazoxan indicating that endogenous NE may occupy high percentage of functional alpha-2 adrenoceptors during resting conditions.

Phenylephrine, except at high concentration, did not influence electrical stimulation-induced [^{3}H]NE release from slices containing raphe nuclei indicating probably lack of alpha-1 adrenoceptors in the regulation of NE release (Fig. **15B**). Moreover, prazosin (0.1 to 1 μM) was also without effect on [^{3}H]NE release. Prazosin was also ineffective on NE release in a microdialysis experiments carried out with probes implanted into the dorsal raphe nuclei of conscious rats [37].

4.3.3. Serotonergic Modulation of Norepinephrine Release in the Raphe Nuclei

The 5-HT_{1A} receptor agonist 8-OH-DPAT added in various concentrations between 1 nM to 1 μM did not influence [^{3}H]NE release from isolated raphe nuclei slices of the rat (Fig. **15B**). The 5-HT_{1A} receptor antagonist WAY-100635 was also without effect on electrical stimulation-induced [^{3}H]NE release. This finding indicates that 5-HT_{1A} receptors are probably not expressed on noradrenergic axon terminals and thus, these receptors are not involved in noradrenergic-serotonergic interaction in the raphe nuclei. Whether other types of 5-HT receptors regulate NE release in the raphe nuclei warrants further elucidation.

Regulation of 5-HT release by 5-HT_{1A} autoreceptors has been discussed in Section 3.1.1.

4.3.4. Serotonergic-Noradrenergic Circuitry in the Raphe Nuclei

Lower brainstem cell groups provide noradrenergic innervation for serotonergic neurons in the raphe nuclei, the noradrenergic influence on serotonergic cells is mediated by alpha-1B and alpha-2A adrenoceptors. However, *in vitro* and *in vivo* experiments resulted in some contradictions in terms how noradrenergic influence occurs. Thus, further experiments need to clarify whether alpha-2 adrenoceptors are located on noradrenergic axon terminals only or on both noradrenergic and serotonergic neurons. Furthermore, albeit alpha-1 adrenoceptors are considered to excite raphe serotonergic neurons, an inhibition of 5-HT release is also observed in some experimental conditions. At last, less is known whether dendritic serotonin release influences the release of NE in the raphe nuclei and that which 5-HT receptors are involved in this neural interaction.

5. REGULATION OF 5-HT RELEASE IN THE PRESENCE MONOAMINE OXIDASE INHIBITORS

It has been shown that both A and B forms of monoamine oxidase (MAO) are present in serotonergic neurons, MAO-B being present in cell bodies of the raphe nuclei and MAO-A migrate to the nerve terminals [18]. Using antiserum directed against MAO-B enzyme led to the conclusion that this enzyme is specifically located in 5-HT-containing neurons and astrocytes in the rat brain [100]. MAO-B enzyme was preferentially detected not only in the rat but also in human raphe nuclei [140].

MAO inhibitors increase extracellular 5-HT concentrations in the raphe nuclei after an acute treatment [19, 40]. The large increase in 5-HT output in the raphe nuclei after systemic administration of MAO inhibitors triggers an activation of 5-HT_{1A} receptors by accumulation of an excess 5-HT in the extracellular space. Acute administration of non-selective MAO inhibitors (phenelzine, tranylcypromine) produces a decrease in 5-HT neuronal firing [25] and reduction of neural firing in the raphe nuclei leads to decreased 5-HT release from the nerve terminal fields [40]. Long-term administration of MAO inhibitors also leads to an elevation of extracellular 5-HT concentrations [41, 59] and repeated injections of MAO inhibitors induces desensitization of 5-somatodendritic HT_{1A} autoreceptors [25, 125]. This desensitization of 5-HT_{1A} autoreceptors is accompanied by recovery of firing of 5-HT neurons.

In axon terminal regions, the release of 5-HT is increased by chronic administration of MAO inhibitors and this effect seems to be independent from changes in somatodendritic 5-HT_{1A} receptor function [26]. This finding indicates that after long-term administration of MAO inhibitors, terminal autoreceptors are probably not desensitized. The MAO-A reversible inhibitor befloxatone enhanced the electrically induced release of [^{3}H]5-HT after a 21-day treatment, whereas the effectiveness of the terminal 5-HT autoreceptor agonist 5-methoxytryptamine was not altered in the hypothalamus, hippocampus and frontal cortex [24].

The selective MAO-A inhibitor clorgyline induces similar changes as nonselective MAO inhibitors. Thus, clorgyline increases extracellular 5-HT concentration in the raphe area. Sustained increase of endogenous 5-HT in clorgyline-treated rats induces desensitization of somatodendritic 5-HT_{1A} autoreceptors [25-27]. Injection of clorgyline for three weeks failed to modify the function of the terminal counterpart as no down-regulation of 5-HT_{1B} receptors occurred. This finding shows that long-term clorgyline treatment also does not alter the function of axon terminal 5-HT autoreceptors.

Less data are available for the selective MAO-B inhibitor L-deprenyl, probably because 5-HT is preferentially deaminated by A form of MAO enzyme [179]. It has been reported that L-deprenyl when administered repeatedly, did not modify the firing activity of 5-HT neurons in the raphe nuclei

[25, 26]. In addition, extracellular 5-HT concentrations in the raphe nuclei or in the frontal cortex were also not altered by doses of L-deprenyl selective for B form MAO enzyme inhibition [40]. However, after complete MAO-A inhibition by clorgyline, administration of L-deprenyl induced a large increase in dialysate 5-HT from the raphe nuclei pointing to the importance of MAO-B in the control of 5-HT release even when MAO-A is inhibited [40]. In our experiments, we have investigated the influence of MAO inhibition on the function of different 5-HT_1 receptor subtypes involved in regulation of 5-HT neurons in the raphe nuclei and found that L-deprenyl desensitizes 5-HT release-mediating 5-HT_{1B} autoreceptors in the rat raphe nuclei leaving 5-HT_{1A} receptor function unaltered.

5.1. Changes in Sensitivity of 5-HT_{1A} and 5-HT_{1B} Receptor Subtypes After Monoamine Oxidase Inhibition

Using superfused raphe nuclei slices preincubated with either clorgyline or L-deprenyl it was found that endogenous 5-HT concentrations increased in response to clorgyline, whereas L-deprenyl did not affect 5-HT content in the midbrain tissue (Table **8**). These data indicate that the selective MAO-A inhibitor clorgyline affects the metabolic degradation of 5-HT in the raphe nuclei but the selective MAO-B inhibitor L-deprenyl does not have this effect. Thus, increase in 5-HT tissue concentrations in raphe nuclei slices superfused with clorgyline indicates an inhibition of type A MAO, this result was not seen after L-deprenyl administration.

Whether selective MAO inhibitors influence 5-HT_{1A} and 5-HT_{1B} receptor sensitivity in 5-HT release inhibition, rat raphe nuclei slices were loaded with [^{3}H]5-HT and superfused with either clorgyline or L-deprenyl. The effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT or the 5-HT_{1B} receptor agonist CGS-12066 were determined on electrically stimulated [^{3}H]5-HT release from clorgyline or L-deprenyl pretreated raphe nuclei slices.

Superperfusion of raphe nuclei slices with L-deprenyl reduced the inhibitory effect of CGS-12066 on electrically stimulated [^{3}H]5-HT release whereas the inhibitory effect of 8-OH-DPAT was still observed (Table **9**). Clorgyline, on the other hand, did not alter the inhibitory effects of either 8-OH-DPAT or CGS-12066 on [^{3}H]5-HT release. These data indicate that *in vitro* administration of L-deprenyl, in conditions where MAO-A enzyme is not inhibited, reduces sensitivity of 5-HT_{1B} autoreceptors to inhibit 5-HT release whereas the activity of 5-HT_{1A} remains unaltered. Neither L-deprenyl nor clorgyline when added to superfused raphe nuclei slices influenced [^{3}H]5-HT release.

5.2. Changes of 5-HT_{1A} and 5-HT_{1B} Receptor Sensitivity in the Raphe Nuclei After Long-Term Treatment with L-Deprenyl

In the next series of experiments, rats were injected either with L-deprenyl (0.25 mg/kg daily s.c.) or saline for 14 days. MAO-A and MAO-B enzyme activities were determined in raphe nuclei tissue [54, 63]. L-Deprenyl inhibited deamination of [^{14}C]5-HT and [^{14}C]phenylethylamine in raphe nuclei tissue by 11% and 75%, respectively. These data demonstrate that sustained administration of small doses of L-deprenyl inhibited B type MAO activity in the raphe nuclei whereas the activity of MAO type A remained substantially unchanged.

The 5-HT_{1A} receptor agonist 8-OH-DPAT decreased the electrically induced [^{3}H]5-HT release from superfused raphe nuclei slices of rats repeatedly injected with L-deprenyl (Table **10**). The 5-HT_{1B} receptor agonist CGS-12066 did not influence, however, [^{3}H]5-HT release in raphe nuclei slices obtained from L-deprenyl-pretreated rats. *In vivo* treatment with L-deprenyl also reduced the inhibitory effect of CGS-12066 on [^{3}H]5-HT release in hippocampal slices (Table **10**). These data demonstrate that not only acute *in vitro* administration of L-deprenyl but also its repeated *in vivo* injections led to selective desensitization of somatodendritic and axon terminal 5-HT_{1B} autoreceptors. Moreover, loss of 5-HT_{1B} receptor sensitivity in 5-HT release inhibition was not accompanied by inhibition of MAO-A enzyme activity.

5.3. Selective Desensitization of 5-HT_{1B} but not 5-HT_{1A} Autoreceptors by L-Deprenyl

Our data indicate that *in vitro* and repeated *in vivo* administration of L-deprenyl reduced sensitivity of 5-HT_{1B} receptors in the raphe nuclei and in the hippocampus without inducing changes in the sensitivity of somatodendritic 5-

Table 8. Effects of *In Vitro* Administration of L-Deprenyl and Clorgyline on 5-HT and 5-Hydroxyindoleacetic Acid (5-HIAA) in Raphe Nuclei Slices of the Rat

Treatments	5-HT	5-HIAA
	(µg/g)	
Non-perfused slices	0.92±0.14	1.39±0.12
Perfused slices	0.47±0.04	0.19±0.02
Slices perfused with L-deprenyl	0.43±0.05	0.07±0.01
Slices perfused with clorgyline	0.86±0.07*	0.07±0.02

Raphe nuclei slices were prepared and superfused with Krebs-bicarbonate buffer. After a 100-min long washout period, L-deprenyl or clorgyline (10 µM) was added to the Krebs-bicarbonate buffer and maintained throughout the experiments for another 100-min period. At the end of superfusion, the slices were collected, weighed, homogenized in 0.4 ml 0.1 M perchloric acid-0.2 mM Nabisulfite and centrifuged. The 5-HT and 5-HIAA contents were measured in the samples by HPLC-electrochemistry [108]. ANOVA and Dunnett's test, *P<0.05, mean±S.E.M., n=4-5.

Table 9. Effects of 5-HT_{1A} and 5-HT_{1B} Receptor Agonists on Electrical Stimulation-Induced [^{3}H]5-HT Release in Raphe Nuclei Slices of the Rat After L-Deprenyl or Clorgyline Pretreatment *In Vitro*

Compounds	Concentration (μM)	[^{3}H]5-HT release (S2/S1)
	Non-treated raphe nuclei slices	
Control	-	0.96±0.06
8-OH-DPAT	1	0.39±0.07*
CGS-12066	1	0.61±0.08*
	L-Deprenyl-treated raphe nuclei slices	
None	-	0.76±0.05
8-OH-DPAT	1	0.30±0.03**
CGS-12066	1	0.77±0.12
	Clorgyline-treated raphe nuclei slices	
None	-	0.95±0.05
8-OH-DPAT	1	0.57±0.08**
CGS-12066	1	0.63±0.06*

Raphe nuclei slices were prepared, loaded with [^{3}H]5-HT and superfused with Krebs-bicarbonate buffer containing clorgyline or the L-deprenyl (10 μM). The slices were superfused with MAO inhibitors for 100 min then 22 3-min fractions were collected. The slices were stimulated electrically (40 V, 2 Hz, 2-msec for 2 min) in fractions 4(S1) and 15(S2) and the release of [^{3}H]5-HT was expressed by the S2/S1 ratio. 8-OH-DPAT and CGS-12066 (1 μM) were added to the buffer from fraction 10 and maintained through the experiment. ANOVA and Dunnett's test, *P<0.05, mean±S.E.M., n=4.

HT_{1A} receptors. This effect is probably not attributed to inhibition of MAO enzyme activity as L-deprenyl did not change 5-HT concentrations in superfused raphe slices and did not modify [^{14}C]5-HT deamination in midbrain tissue after long-term administration. These data further indicate that the attenuated function of 5-HT_{1B} autoreceptors evoked by L-deprenyl is not a consequence of an increased availability of 5-HT in the synaptic cleft. Moreover, acute clorgyline, which increased tissue concentrations and probably also synaptic availability of 5-HT did not induce changes in sensitivity of 5-HT_{1A} and 5-HT_{1B} receptor subtypes. It has been reported, however, that repeated administration of clorgyline is required for detection of changes in somatodendritic 5-HT_{1A} receptor function [28].

There might be several possible explanations for the mechanism by which L-deprenyl selectively modifies 5-HT_{1B} autoreceptor functions. An interaction between inhibition of neuronal uptake and inhibition of neurotransmitter release through the effect of an agonist acting on autoreceptors was already reported [65, 99, 122]. It is possible to speculate therefore that the 5-HT uptake inhibitory effect of L-deprenyl [35, 74] may have a role in reducing the inhibitory effect of CGS-12066 on [^{3}H]5-HT release. The effect of L-deprenyl-induced 5-HT uptake blockade on 5-HT_{1B} receptor-mediated [^{3}H]5-HT release inhibition may indicate a close location of the 5-HT transporter to the autoreceptor organized in a serotonergic synapse. 5-HT transporter and 5-HT_{1A} autoreceptors, on the other hand, are probably not in a

Table 10. Effects of 5-HT_{1A} and 5-HT_{1B} Receptor Agonists on Electrical Stimulation-Induced [^{3}H]5-HT Release from Raphe Nuclei and Hippocampal Slices Dissected from Rats Repeatedly Treated with L-Deprenyl

Compounds	Concentration (μM)	[^{3}H]5-HT release (S2/S1)	
		Raphe nuclei	Hippocampus
Saline treatment	-	1.18±0.21	0.95±0.03
L-Deprenyl treatment	-	1.15±0.14	1.01±0.08
+ 8-OH-DPAT	1	0.49±0.11*	0.79±0.05
+ CGS-12066	1	0.81±0.09	1.17±0.14

Slices containing raphe nuclei or hippocampus were prepared from rat, which had been repeatedly treated with 0.25 mg/kg of L-deprenyl sc for 14 days. The slices were loaded with [^{3}H]5-HT, superfused and stimulated electrically (40 V, 2 Hz, 2-msec for 2 min) in fractions 4(S1) and 15(S2). The release of [^{3}H]5-HT was expressed by the S2/S1 ratio. Agonists were added 9 min before S2. ANOVA and Dunnett's test, *P<0.05, mean±S.E.M., n=3-4.

close link as somatodendritic 5-HT_{1A} receptors may have a preferential role in regulation of non-synaptic 5-HT release and the transporter may not be in a close vicinity of the receptor.

It has been shown that [^{3}H]imipramine binding site and terminal 5-HT autoreceptors are interactive and activation of this site leads to decrease autoreceptor function in regulation of 5-HT release [99]. Since L-deprenyl has been reported to increase the number of [^{3}H]imipramine recognition site [181] the possibility arises that interaction exists between L-deprenyl bound to 5-HT uptake site and activity of 5-HT_{1B} autoreceptors. The decreased function of 5-HT_{1B} autoreceptors in response to L-deprenyl-treated rats appears to result from a functional interaction between this type of release-mediating autoreceptor and an effect on [^{3}H]imipramine binding sites.

Besides MAO inhibitors, 5-HT reuptake blockers when administered in a long-term, also exert profound effects on the sensitivity of the release-mediating 5-HT_1 autoreceptors in the somatodendritic area as well as in the axon terminals. It has been shown that sustained administration of paroxetine induces desensitization 5-HT_{1A} autoreceptors and also decreases effectiveness of 5-HT_{1D} receptor activation in reducing 5-HT release from rat raphe nuclei slices [125]. Desensitization of 5-HT_{1D} receptors in mesencephalic raphe and in hippocampus of the guinea pig may also occur after long-term paroxetine administration [55]. Repeated administration of 5-HT reuptake blockers leads to desensitization of terminal 5-$HT_{B/1D}$ autoreceptors in different 5-HT projection areas and this was indicated by a reduced efficacy of 5-$HT_{1B/1D}$ agonists to inhibit 5-HT release [126]. In another series of experiments, the 5-HT uptake inhibitor fluvoxamine, after sustained administration, was found to induce desensitization of somatodendritic 5-HT_{1A} receptors but not of 5-$HT_{1B/1D}$ terminal autoreceptors [17]. Our experiments provide evidence that, in contrast to 5-HT reuptake inhibitors, L-deprenyl evokes desensitization of central and peripheral 5-$HT_{1B/1D}$ receptors leaving the sensitivity of 5-HT_{1A} receptors unaltered.

CONCLUSION

In the raphe nuclei, 5-HT is released from serotonergic projection neurons as action potential propagation invades dendrites, recurrent axon collaterals or axon terminals. This release may occur from vesicles by exocytosis or from cytoplasmic pool with the mechanism of reverse-mode operation of neurotransmitter transporters. 5-HT release in the raphe nuclei may take place in synaptic organizations or directly into the biophase and thus, both synaptic and non-synaptic neurotransmission are present.

The release of 5-HT in the raphe nuclei is regulated by various 5-HT receptors. These receptors may have various locations in central part of serotonergic neurons: the release- and impulse-mediating 5-HT_{1A} autoreceptors may be located on the somatodendritic part; the release-inhibitory 5-$HT_{1B/1D}$ receptor may be on recurrent axon collaterals and axon terminals. The subtypes of 5-HT_1 receptors mediate feedback inhibition of 5-HT release. 5-HT_1 receptor subtypes can exert both synaptic and non-synaptic influences on 5-HT release. 5-$HT_{1B/1D}$ receptors may be under tonic influence of 5-HT released whereas 5-HT_{1A} receptors may influence raphe 5-HT release with a more phasic mode.

The selectivity of 5-HT_1 receptor subtypes was differently altered by L-deprenyl, a selective inhibitor of B type MAO. Thus, acute *in vitro* and repeated *in vivo* administration of L-deprenyl reduced sensitivity of 5-HT_{1B} receptors in the raphe nuclei and the hippocampus without inducing changes in the sensitivity of somatodendritic 5-HT_{1A} receptors. This effect is probably not attributed to inhibition of MAO enzyme activity but rather to 5-HT uptake inhibitory effect of L-deprenyl. The inhibitory effects L-deprenyl on 5-HT carrier molecules and 5-$HT_{1B/1D}$ receptors may indicate a close location of 5-HT transporter and autoreceptor organized in serotonergic synapses.

Evidence was presented for the involvement of the ionotropic 5-HT_3 receptors in mediation of raphe 5-HT release. 5-HT_3 receptors stimulate exocytotic vesicular release of 5-HT and that mediated by reverse mode operation of 5-HT transporter. The release-facilitatory 5-HT_3 receptors may be sited on the proximal dendrites, dendritic shaft or on cell body with an extrasynaptic organization. 5-HT_3 receptors mediate feedback stimulation of raphe 5-HT release.

There may be an integrated operation of 5-HT receptors in the regulation of 5-HT release in the raphe nuclei (Fig. **16**). As 5-HT release occurs, the subtypes of 5-HT_1 autoreceptors in the dendrites, axon collaterals and nerve endings are activated leading to inhibition the further release of 5-HT. If the autoreceptor-mediated inhibition is attenuated (autoreceptor is preoccupied by 5-HT or 5-HT transporter is down-regulated) or initiated with a delay, the concentration of 5-HT in the gap of dendritic synapse may reach high enough concentrations to diffuse out into the extrasynaptic space. 5-HT traveling a considerable distance in the biophase may activate 5-HT_3 receptors located extrasynaptically and as a consequence, changes in electrical properties of dendritic membranes will occur. 5-HT_3 receptors may be located more proximally on dendritic tree and therefore, depolarization of the membranes will not allow propagation of inhibitory inputs from distal dendrites to the dendritic shaft and cell body. GABA may be a possible candidate for this kind of inhibition as GABAergic neurons in the raphe nuclei are known to synapse with serotonergic neurons [62]).

Another possible consequence of 5-HT_3 receptor stimulation in the raphe nuclei is an increase of 5-HT release originated either from the vesicular or the cytoplasmic pools or both of serotonergic dendrites. The facilitation of 5-HT release by non-synaptic 5-HT_3 receptors will result in high 5-HT concentrations in the extrasynaptic space leading to transmitter diffusion toward 5-HT-containing dendrites and recurrent axon collaterals equipped with inhibitory 5-HT_{1A} and 5-$HT_{B/D}$ autoreceptors. These dendrites or nerve endings will then be hyperpolarized and not be invaded by action potential and 5-HT release will be inhibited. Therefore, facilitation of 5-HT release by extrasynaptic 5-HT_3 receptors may induce release-inhibition from a large number of dendrites or recurrent axon collaterals (Fig. **16**). The facilitatory 5-HT_3 and inhibitory 5-HT_1 receptor subtypes exert opposing role in the control of somatodendritic 5-HT release. Thus,

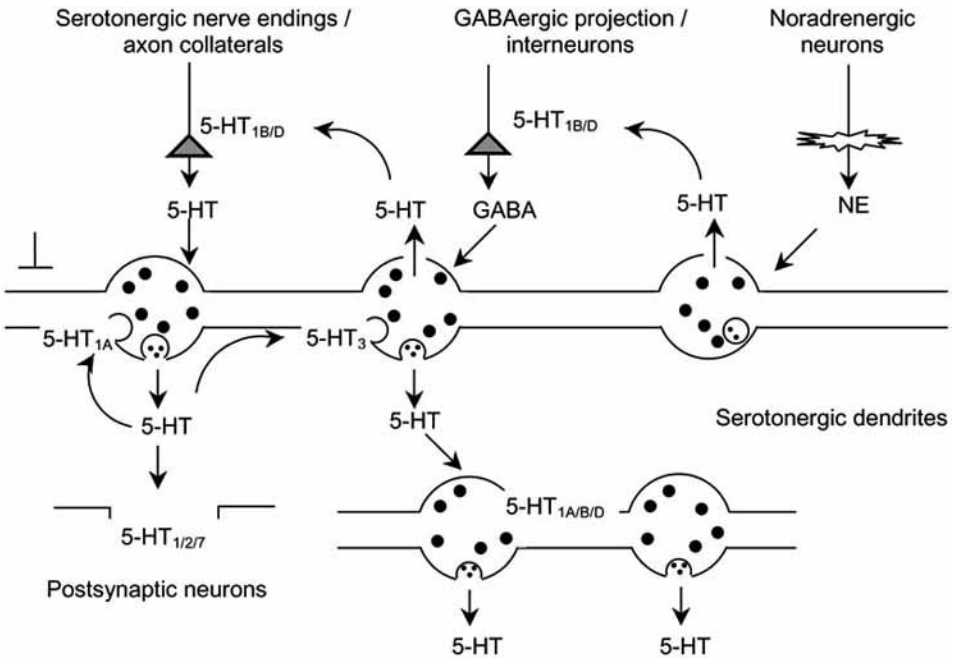

Fig. (16). Possible neuronal interaction in the raphe nuclei. 5-HT may be released into synapses formed between vesicle-containing dendrites of serotonergic neurons and impinging neurons. 5-HT release in dendritic synapses is inhibited by presynaptic 5-$HT_{1B/1D}$ autoreceptors. 5-HT released into the synaptic cleft will activate postsynaptic 5-HT heteroreceptors (inhibitory: 5-HT_1, 5-HT_7 excitatory: 5-HT_2). If once released, 5-HT diffuses out from the synaptic cleft into the biophase and it may reach extrasynaptic 5-HT_3 receptors. 5-HT spilled over from the synapses or released non-synaptically from the dendrites will facilitate 5-HT_3 receptors. The transmitter release occurring by reverse-mode operation of 5-HT transporter or by exocytosis will further increase 5-HT concentrations in the extrasynaptic space. As a consequence, dendritic membranes around 5-HT_3 receptors will be depolarized and GABAergic inhibitory synaptic inputs from distal dendrites will not propagate proximally to the dendritic shaft and cell body of serotonergic neurons. Furthermore, high concentrations of 5-HT in the biophase will activate a number of inhibitory 5-$HT_{1B/1D}$ receptors sited on serotonergic recurrent axon collaterals and 5-HT_{1A} receptors on parallel dendrites. This will lead to inhibition of 5-HT release in a large number of serotonergic dendritic branches and axon terminals.

there may be an interaction between inhibitory 5-HT_1 and facilitatory 5-HT_3 receptors in the regulation of somatodendritic 5-HT release in the raphe nuclei.

The local neuronal circuit in the raphe nuclei consists of serotonergic projection neurons, GABAergic interneurons and glutamatergic axon terminals. 5-HT inhibits GABA release in synaptic or non-synaptic communications and both 5-HT_{1A} and 5-$HT_{1B/1D}$ receptors are involved in this inhibition. The location of the two 5-HT_1 receptor subtypes may be, however, different: 5-$HT_{1B/1D}$ heteroreceptors are probably located on GABAergic axon terminals and they exert a direct inhibition on GABA release. 5-HT_{1A} receptors that inhibit GABA release are probably those located on 5-HT cells and mediate autoinhibition of 5-HT release. Thus, inhibition of raphe GABA release by 5-HT_{1A} receptors is due to an indirect effect.

GABA, if once released from axon terminals of interneurons or projection neurons, inhibits 5-HT release by stimulating $GABA_A$ and $GABA_B$ receptors located on serotonergic projection neurons. $GABA_B$ receptors may mediate a tonic inhibition whereas $GABA_A$ receptors exert a more phasic inhibition on 5-HT release. These data indicate a reciprocal interaction between serotonergic and GABAergic neurotransmission in the raphe nuclei. Moreover, reciprocal innervation may exist between serotonergic and glutamatergic nerve endings; 5-HT released into the biophase may inhibit glutamate release either in a synaptic or non-synaptic communication by 5-HT_7 heteroreceptors located on glutamatergic axon terminals.

Activation of 5-HT_7 heteroreceptors on glutamatergic axon terminals by 5-HT inhibits glutamate release in the raphe nuclei. This inhibition reduces excitatory influence to serotonergic neurons, which then leads to inhibition of 5-HT release. 5-HT released from projection neurons exerts dual inhibitory and excitatory influences on glutamate release; these effects may be mediated by 5-HT_7 and 5-HT_2 receptors expressed on glutamatergic nerve endings. The postulated glutamatergic-serotonergic interaction in the raphe nuclei was further evidenced by the finding that NMDA and AMPA, agonists for ionotropic glutamatergic receptors, enhance 5-HT release in raphe nuclei slices.

Within the raphe nuclei, serotonergic projection neurons and axon terminals of noradrenergic neurons from the locus coeruleus are in reciprocal interactions at the somatodendritic and axon terminal levels. The noradrenergic influence of serotonin cells is mediated by pre- and postsynaptic alpha-2 and alpha-1 heteroceptors, which regulate cell firing as well as 5-HT and NE release. Serotonergic-noradrenergic interplay in the raphe nuclei is an important target for a number of psychoactive drugs, including selective and non-

selective serotonin reuptake inhibitors, MAO inhibitors and atypical antipsychotic drugs [31]. Several clinical observations conclude that neuronal network of the raphe nuclei mediates a number of psychiatric disorders. Ascending serotonergic pathways originating from the raphe nuclei and projecting to the amygdala and frontal cortex or to the dorsal periaqueductal gray matter have been implicated in the pathology of generalized anxiety disorder and panic disorder, respectively [127]. Of the various 5-HT receptors, 5-HT_{1A} and 5-HT_{2A} receptors were suggested to be involved in development of various anxietic disorders. Evidence for reduced serotonergic input from the dorsal raphe nuclei to the prefrontal cortex has been reported in patients suffering from major depression or attempted suicide [34, 96]. We believe that detailed investigation of neural network and neurotransmitter interactions within the raphe nuclei may lead not only to better understanding drugs' mode of action but also to discover clinically potent new compounds for treatment of psychiatric disorders.

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REFERENCES

[1] Abellan, M. T., Jolas, T., Aghajanian, G. K., Artigas, F. (2000) Dual control of dorsal raphe serotonergic neurons by $GABA_B$ receptors. Electrophysiological and microdialysis studies. *Synapse,* **36**, 21-34.

[2] Abrams, J. K., Johnson, P. L., Hollis, J. H., Lowry, C. (2004) Anatomic and functional topography of the dorsal raphe nucleus. *Annals New York Academy of Sciences,* **1018**, 46-57.

[3] Adell, A., Artigas, F. (1999) Regulation of the release of 5-hydroxytryptamine in the median raphe nucleus of the rat by catecholaminergic afferents. *Eur. J. Neurosci.,* **11**, 2305-2311.

[4] Adell, A., Celeda, P., Artigas, F. (2001) The role of 5-HT_{1B} receptors in the regulation of serotonin cell firing and release in the rat brain. *J. Neurochem.,* **79**, 172-182.

[5] Aghajanian, G. K., Wang, R. Y. (1977) Habenular and other midbrain raphe afferents demonstarted by a modified retrograde tracing technique. *Brain Res.,* **122**, 229-242.

[6] Aghajanian, G. K., Marek, G. J. (2000) Serotonin model of schizophrenia: emerging role of glutamate mechanisms. *Brain Res. Rev.,* **31**, 302-312.

[7] Arborelius, L., Backlund Hook, B., Hackell, U., Svensson, T. H. (1994) The 5-HT_{1A} receptor antagonist (S)-UH-301 blocks the (R)-8-OH-DPAT-induced inhibition of serotonergic dorsal raphe cell firing in the rat. *J. Neural. Transm.,* **96**, 179-186.

[8] Bagdy, E., Horvath, E., Sziraki, I., Kiraly, I., Harsing, L. G., Jr. (1994) Further evidence on 5-HT_{1A} agonist action of 8-OH-DPAT, *in vitro* release studies. 17th Annual Meeting of the European Neuroscience Association Abstr., **51**, 19.

[9] Bagdy, E., Harsing, L. G., Jr. (1995) The role of various calcium and potassium channels in the regulation of somatodendritic serotonin release. *Neurochem. Res.,* **20**, 1409-1415.

[10] Bagdy, E., Harsing, L. G., Jr. (1997) Opposing role of 5-HT_{1A} and 5-HT_3 receptors in the regulation of somatodendritic 5-HT release. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **356**, R54.

[11] Bagdy, E., Solyom, S., Harsing, L. G., Jr. (1998) Feedback stimulation of somatodendritic serotonin release: a 5-HT_3 receptor-mediated effect in the raphe nuclei of the rat. *Brain Res. Bulletin,* **45**, 203-208.

[12] Bagdy, E., Harsing, L. G., Jr., Kiraly, I. (2000) Reciprocal innervation between serotonergic and GABAergic neurons in the raphe nuclei of the rat. *Neurochem. Res.,* **25**, 1465-1473.

[13] Baraban, J. M., Aghajanian, G. K. (1981) Noradrenergic innervation of serotonergic neurons in the dorsal raphe: Demonstration by electron microscopic autoradiography. *Brain Res.,* **204**, 1-11.

[14] Bard, J. A., Zgombick, J., Adham, N., Vaysee, P., Branchek, T. A., Weinshank, R. L. (1993) Cloning of a novel human serotonin receptor (5-HT_7) positively linked to adenylate cyclase. *J. Biol. Chem.,* **268**, 23422-23426.

[15] Barnes, N. M., Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology,* **38**, 1083-1152.

[16] Behzadi, G., Kalen, P., Parvapassu, F., Wiklund, L. (1990) Afferents to the median raphe nucleus of the rat: retrograde choleratoxin and wheat germ-conjugated horseradish peroxidase tracing and selective D-[^{3}H]aspartate labelling of possible excitatory amino acid inputs. *Neuroscience,* **37**, 77-100.

[17] Bel, N., Artigas, F. (1993) Chronic treatment with fluvoxamine increases extracellular 5-HT in frontal cortex but not in raphe nuclei. *Synapse,* **15**, 243-245.

[18] Bel, N., Artigas, F. (1995) *In vivo* evidence for the reversible action of the monoamine oxidase inhibitor brofaromine on 5-hydroxytryptamine release in rat brain. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **351**, 475-482.

[19] Bel, N., Artigas, F. (2005) Fluvoxamine preferentially increases extracellular 5-hydroxytryptamine in the raphe nuclei: An *in vivo* microdialysis study. *Eur. J. Pharmacol.,* **229**, 101-103.

[20] Belin, M. F., Augera, M., Tappaz, M., McRae-Degueurce, A., Bobillier, P., Pujol, J. F. (1979) GABA-accumulating neurons in the nucleus raphe dorsalis and periaqueductal gray in the cat: biochemical and radioautographic study. *Brain Res.,* **170**, 279-297.

[21] Bengel, D., Murphy, D. L., Andrews, A. M., Wichems, C. H., Feltner, D., Heils, A., Mossner, R., Westphal, H., Lesch, K. P. (1998) Altered brain serotonin homeostasis and locomotor insensitivity to 3,4-methylenedioxymethamphetamine ("ecstasy") in serotonin transporter-deficient mice. *Mol. Pharmacol.,* **53**, 649-655.

[22] Benuck, M., Reith, M. E. A. (1992) Dopamine releasing effect of phenylbiguanide in rat striatal slices. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **345**, 666-672.

[23] Bie, B., Fields, H. L., Williams, J. T., Pan, Z. Z. (2003) Roles of alpha1- and alpha2-adrenoceptors in the nucleus raphe magnus in opioid analgesia and opioid abstinence-induced hyperalgesia. *J. Neurosci.,* **23**, 7950-7957.

[24] Blier, P., Bouchard, C. (1984) Modulation of 5-HT release in the guinea-pig brain following long-term administration of antidepressant drugs. *Br. J. Pharmacol.,* **113**, 485-495.

[25] Blier, P., de Montigny, C. (1985) Serotonergic but not noradrenergic neurons in rat central nervous system adapt to long-term treatment with monoamine oxidase inhibitors. *Neuroscience,* **16**, 949-955.

[26] Blier, P., de Montigny, C., Azzaro, A. J. (1986) Modification of serotonergic and noradrenergic neurotransmissions by repeated administration of monoamine oxidase inhibitors: Electrophysiological studies in the rat central nervous system. *J. Pharm. Exp. Ther.,* **237**, 987-994.

[27] Blier, P., de Montigny, C., Azzaro, A. J. (1986) Effect of repeated amiflamine administration on serotonergic and noradrenergic neurotransmission: Electrophysiological studies in the rat CNS. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **334**, 253-260.

[28] Blier, P., Chaput, Y., de Montigny, C. (1988) Long-term 5-HT reuptake blockade,but not monoamine oxidase inhibition,decreases the function of terminal 5-HT autoreceptors: an electrophysiological study in the rat brain. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **337**, 246-254.

[29] Blier, P., Bouchard, C. (1993) Functional characterization of a 5-HT_3 receptor which modulates the release of 5-HT in the guinea-pig brain. *Br. J. Pharmacol.,* **108**, 13-22.

[30] Blier, P., Monroe, P. J., Bouchard, C., Smith, D. L., Smith, D. J. (1993) 5-HT_3 receptors which modulate [^{3}H]5-HT release in the guinea-pig hypothalamus are not autoreceptors. *Synapse,* **15**, 143-148.

[31] Blier, P., Szabo, S. T. (2005) Potential mechanisms of action of atypical antipsychotic medication in treatment-resistant depression and anxiety. *J. Clin. Psychiatry*, **66**, 30-40.

[32] Boddeke, H. W. G. M., Fargin, A., Raymond, J. R., Schoeffter, P., Hoyer, D. (1992) Agonist/antagonist interactions with cloned human 5-HT_{1A} receptors: Variations in intrinsic activity studies in transfected HeLa cells. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **345**, 257-263.

[33] Boess, F. G., Martin, I. L. (1994) Molecular biology of 5-HT receptors. *Neuropharmacology,* **33**, 275-317.

[34] Boldrini, M., Underwood, M. D., Mann, J. J., Arabgo, V. (2005) More tryptophan hydroxylase in the brainstem dorsal raphe nucleus in depressed suicides. *Brain Res.,* **1041**, 19-28.

[35] Bondioletti, G. P., Galva, M. D., Villa, F., Sciaba, L., Picotti, G. B. (1995) *In vitro* effects on monoamine uptake and release by the reversible monoamine oxidase-B inhibitors lazabemide and N-(2-aminoethyl)-p-chlorobenzamide: a comparison with L-deprenyl. *Biochem. Pharmacol.,* **50**, 97-102.

[36] Boothman, L. J., Allers, K. A., Rasmussen, K., Sharp, T. (2003) Evidence that central 5-HT_{2A} and 5-$HT_{2B/2C}$ receptors regulate 5-HT cell firing in the dorsal raphe nucleus of the anaesthetised rat. *Br. J. Pharmacol.,* **139**, 998-1004.

[37] Bortolozzi, A., Artigas, F. (2003) Control of 5-hydroxytryptamine release in the dorsal raphe nucleus by the noradrenergic system in rat brain. *Neuropsychopharmacology*, **28**, 421-434.

[38] Bourdon, D. M., Camden, J. M., Landon, L. A., Levy, F. O., Turner, J. T. (2000) Identification of the adenylyl cyclase-activating 5-hydroxytryptamine receptor subtypes expressed in the rat submandibular gland. *Br. J. Pharmacol.,* **130**, 104-108.

[39] Boyland, P. S., Eastwood, S., Ekkis, C., Bergsma, D., Jones, B. J., Gloge, S., Upton, N., Middlemniss, D. N. (1996) High specific activity [^{3}H]5-CT binding: correlation of guinea-pig cortex with human cloned 5-HT_7 receptors. *Br. J. Pharmacol.,* **117**, 132P.

[40] Celada, P., Artigas, F. (1993) Monoamine oxidase inhibitors increase preferentially extracellular 5-hydroxytryptamine in the midbrain raphe nuclei. A brain microdialysis study in the awake rat. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **347**, 583-590.

[41] Celada, P., Bel, N., Artigas, F. (1994) The effects of brofaromine,a reversible MAO-A inhibitor,on extracellular 5-HT in the raphe nuclei and frontal cortex of freely moving rats. *J. Neural. Transm.,* **41**, 357-363.

[42] Celada, P., Puig, M. V., Casanovas, J. P., Guillazo, G., Artigas, F. (2001) Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: involvement of serotonin-1A,$GABA_A$ and glutamate receptors. *J. Neurosci.,* **21**, 9917-9929.

[43] Chazal, G., Ralston, H. J. II. (1987) 5-HT-containing structures in the nucleus raphe dorsalis of the cat: an ultrastructural analysis of dendrites,presynaptic dendrites,and axon terminals. *J. Comp. Neurol.,* **259**, 259-317.

[44] Cliffe, I. A., Brightwell, C. I., Fletcher, A., Forster, E. A., Mansell, H. L., Reilly, Y., Routledge, C., White, A. C. (1993) (S)-N-tert-Butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2- phenylpropanamide [(S)-WAY-100135]: a selective antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors. *J. Med. Chem.,* **36**, 1509-1510.

[45] Cook, N. (1988) The pharmacology of potassium channels and their therapeutic potential. *Trends Pharmacol. Sci.,* **9**, 21-28.

[46] Cornea-Hebert, V., Riad, M., Wu, C., Singh, S. K., Descarries, L. (1999) Cellular and subcellular distribution of the serotonin 5-HT_{2A} receptor in the central nervous system of adult rat. *J. Comp. Neurol.,* **409**, 187-209.

[47] Craven, R., Grahame-Smith, D., Newberry, N. (1994) WAY-100635 and GR 127935: Effects on 5-hydroxytryptamine-containing neurones. *Eur. J. Pharmacol.,* **271**, R1-R3.

[48] Dahlstrom, A., Fuxe, K. (1964) Evidence for the existence of monoamine containing neurons in the central nervous system 1. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.* Suppl., **232**, 1-55.

[49] Davidson, C., Stamford, J. A. (1995) Evidence that 5-hydroxytryptamine release in rat dorsal raphe nucleus is controlled by 5-$HT_{1A,}$ 5-$HT_{1B\ and}$ 5-HT_{1D} autoreceptors. *Br. J. Pharmacol.,* **114**, 1107-1109.

[50] Day, H. E., Campeau, S., Watson, S. J., Jr., Akil,H. (1997) Distributionof alpha 1a-, alpha 1b- and alpha 1d-adrenergic receptor mRNA in the rat brai n and spinal cord. *J. Chem. Neuroanat*., **13**: 115-139.

[51] de Langen, C. D. J., Mulder, A. H. (1979) Compartmental analysis of the accumulation of ^{3}H-dopamine in synaptosomes from rat corpus striatum. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **308**, 31-39.

[52] Derkach, V., Surprenant, A., North, R. A (1989) 5-HT_3 receptors are membrane ion channels. *Nature,* **339**, 706-709.

[53] Duncan, M. J., Temel, S., Jennes, L. (2001) Localization of serotonin 5-HT_7receptor immunoreactivity in the rat brain. *Soc. Neurosci.* .

[54] Ekstedt, B. (1976) Substrate specificity of the different forms of monoamine oxidase in rat liver mitochondria. *J. Biochem. Pharm.,* **25**, 1133-1138.

[55] El Mansari, M., Blier, P. (1996) Functional characterization of 5-HT_{1D} autoreceptors on the modulation of 5-HT release in the guinea-pig mesencephalic raphe,hippocampus and frontal cortex. *Br. J. Pharmacol.,* **118**, 681-689.

[56] Engel, G., Gothert, M., Hoyer, D., Schlicker, E., Hillenbrand, K. (1986) Identify of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat cortex with 5-HT_{1B} binding sites. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **332**, 1-7.

[57] Erlander, M. G., Lovenberg, T. W., Baron, B. M., De Lecea, L., Danielson, P. E., Racke, M., Slone, A. L., Siegel, B. W., Foye, P. E., Cannon, K., Burns, J. E., Sutcliffe, J. G. (1993) Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. *Proc. Natl. Acad. Sci. USA,* **52**, 949-958.

[58] Ferraro, G., Montalbano, M. E., Sardo, P., La Grutta, V. (1996) Lateral habenular influence on dorsal raphe neurons. *Brain Res. Bull.,* **41**, 47-52.

[59] Ferrer, A., Arigas, F. (1994) Effects of single and chronic treatment with tranylcypromine on extracellular 5-HT in rat brain. *Eur. J. Pharmacol.,* **263**, 227-234.

[60] Fletcher, A., Forster, E. A., Bill, D. J., Brown, G., Cliffe, I. A., Hartley, J. E., Jones, D. E., McLenachan, A., Stanhope, K. J., Critchley, D. J. P., Childs, K. J., Middlefell, V. C., Lanfumey, L., Corradetti, R., Laporte, A., Gozlan, H., Hamon, M., Dourish, C. T. (1996) Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635,a potent,selective and silent 5-HT_{1A} receptor antagonist. *Behav. Brain Res.,* **73**, 337-353.

[61] Forbes, I. T., Dabbs, S., Duckworth, D. M., Jennings, A. J., King, F. D., Lovell, P. J., Brown, A. M., Collin, L., Hagan, J. J., Middlemniss, D. N., Riley, G. J., Thomas, D. R., Upton, N. (1998) (R)-3,N-dimethyl-N-[1-methyl-3-(4-methyl-piperiodin-1-y)propyl]benzenesulfonamide: the first selective 5-HT_7 receptor antagonist. *J. Med. Chem.,* **41**, 655-657.

[62] Forchetti, C. M., Meek, J. L. (1981) Evidence for a tonic GABAergic control of serotonin neurons in the median raphe nucleus. *Brain Res.,* **206**, 208-212.

[63] Fowler, C. J., Strolin Benedetti, M. (1983) The metabolism of dopamine by both forms of monoamine oxidase in the rat brain and its inhibition by cimoxaton. *J. Neurochem.,* **40**, 1534-1541.

[64] Gallager, D. W., Aghajanian, G. K. (1976) Effect of antipsychotic drugs on the firing of dorsal raphe cells. II. Reversal by picrotoxin. *Eur. J. Pharmacol.,* **39**, 357-364.

[65] Galzin, A. M., Moret, C., Verzier, B., Langer, S. Z. (1985) Interaction between tricyclic and nontricyclic 5-hydroxytryptamine uptake inhibitors and the presynaptic 5-hydroxytryptamine inhibitory autoreceptors in the rat hypothalamus. *J. Pharm. Exp. Ther.,* **235**, 200-211.

[66] Galzin, A. M., Poncet, V., Langer, S. Z. (1990) 5-HT_3 receptor agonists enhance the electrically-evoked release of [3H]-5-HT in guinea-pig frontal cortex slices. *Br. J. Pharmacol.,* **101**, 307P.

[67] Gustafson, E. L., Durkin, M. M., Bard, J. A., Zgombick, J., Branchek, T. A. (1996) A receptor autoradiographic and *in situ* hybridization analysis of the distribution of the 5-HT_7 receptor in rat brain. *Br. J. Pharmacol.,* **117**, 657-666.

[68] Guyenet, P. G., Stornetta, R. L., Riley, T., Norton, F. R., Rosin, D. L., Lynch, K. R. (1994) Alpha 2A-adrenergic receptors are present in lower barinstem catecholaminergic and serotonergic neurons innervating spinal cord. *Brain Res.,* **638**, 285-294.

[69] Hagan, J. J., Price, G. W., Jeffrey, P., Deeks, N. J., Stean, T., Piper, D., Smith, M. I., Upton, N., Medhurst, A. D., Middlemniss, D. N., Riley, G. J., Lovell, P. J., Bromidge, S. M., Thomas, D. R. (2000) Characterization of SB-269970-A, a selective 5-HT_7 receptor antagonist. *Br. J. Pharmacol.,* **130**, 539-548.

[70] Haj-Dahmane, S., Hamon, M., Lanfumey. L. (1991) K^+ channel and 5-hydroxytryptamine 1A autoreceptor interactions in the rat dorsal raphe nucleus: an *in vitro* electrophysiological study. *Neuroscience,* **41**, 495-505.

[71] Hajos, M., Richards, C. D., Szekely, A. D., Sharp, T. (1998) An electrophysiological and neuroanatomical study of the medial prefrontal cortical projection to the midbrain raphe nuclei in the rat. *Neuroscience,* **87**, 95-108.

[72] Hamblin, M. W., McGuffin, R. W., Metcalf, M. A., Dorsa, D. M., Merchant, K. M. (1992) Distinct 5-HT_{1B} and 5-HT_{1D} serotonin receptors in rat: structural and pharmacological characterization of the two cloned receptors. *Mol. Cell Neurosci.,* **3**, 578-587.

[73] Harandi, M., Aguera, M., Gamrani, H., Didier, M., Maitre, M., Calas, A., Belin, M. F. (1987) y-Aminobutyric acid and 5-hydroxytryptamine interrelationship in the rat nucleus raphe dorsalis: combination of radiographic and immunocytochemical techniques at light and electron microscopy levels. *Neuroscience,* **21**, 237-251.

[74] Harsing, L. G., Jr., Tekes, K., Magyar, K., Knoll, J. (1980) Deprenyl inhibits dopamine uptake in the rat striatum, in: Magyar, K., Knoll, J. Eds, Monoamine Oxidases and Their Selective Inhibition. Budapest, Pergamin Press-Akademiai Kiado, pp. 45-56.

[75] Harsing, L. G. Jr., Sershen, H., Lajtha, A. (1992) Dopamine efflux from striatum after chronic nicotine: evidence for autoreceptor desensitization. *J. Neurochem.,* **59**, 48-54.

[76] Harsing, L. G., Jr., Zigmond, M. J. (1997) Influence of dopamine on GABA release in striatum: evidence for D1-D2 interactions and non-synaptic influences. *Neuroscience,* **77**, 419-429.

[77] Harsing, L. G., Jr., Bagdy, E., Kiraly, I., Csillik-Perczel, V., Sebestyen, L. (2000) L-Deprenyl desensitizes 5-HT_{1B} but not 5-HT_{1A} serotonin release-mediating autoreceptors in the raphe nuclei of the rat, in: Magyar, K., Vizi, E. S. Eds, Milestones in monoamine oxidase research: discovery of (-)deprenyl Budapest, Medicina Publishing House Co, pp. 107-130.

[78] Harsing, L. G. Jr., Kiraly, I., Bagdy, E. (2000) Signal transduction pathways coupled to serotonin release-inhibitory 5-HT_{1A} and 5-HT_{1B} receptors in rat raphe nuclei slices. *J. Physiol. Proceedings,* **526**, 67P.

[79] Harsing, L. G. Jr., Juranyi, Zs. (2004) Evidence for a reciprocal serotonergic-glutamatergic interaction in the raphe nuclei: involvement of $5HT_7$ receptors. *Australian Neurosci. Soc. Abstr. 120*.

[80] Harsing, L. G. Jr., Prauda, I., Barkoczy, J., Matyus, P., Juranyi, Zs. (2004) 5-HT_7 heteroreceptor-mediated inhibition of [^{3}H]serotonin release in raphe nuclei slices of the rat: evidence for a serotonergic-glutamatergic interaction. *Neurochem. Res.,* **29**, 1479-1489.

[81] Hervas, I., Queiroz, C. M., Adell, A., Artigas, F. (2000) Role of uptake inhibition and autoreceptor activation in the control of 5-HT release in the frontal cortex and dorsal hippocampus of the rat. *Br. J. Pharmacol.,* **130**, 160-166.

[82] Hery, F., Faudon, M., Ternaux, J. P. (1982) *In vivo* release of 5-HT in two raphe nuclei (raphe dorsalis and magnus) of cat. *Brain Res.,* **8**, 123-129.

[83] Hjorth, S., Magnusson, T. (1988) The 5-HT_{1A} receptor agonist,8-OH-DPAT,preferentially activates cell body 5-HT autoreceptors in rat brain *in vivo*. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **338**, 463-471.

[84] Hopwood, S. E., Stamford, J. A. (2001) Noradrenergic modulation of serotonin release in rat dorsal and median raphe nuclei *via* alpha(1) and alpha(2A) adrenoceptors. *Neuropharmacology,* **41**, 433-442.

[85] Hoyer, D., Clarke, D. E., Fozard, J. E., Hartig, P. R., Martin, G. R., Mylecharane, E. J., Saxena, P. R., Humbrey, P. P. A. (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.,* **46**, 157-204.

[86] Hoyer, D., Hannon, J. P., Martin, G. R. (2002) Molecular,pharmacological and functional diversity of 5-HT receptors. *Pharm. Biochem. Behav.,* **71**, 533-554.

[87] Hu, P. -S., Fredholm, B. B. (1989) Alpha2-adrenoceptor agonist-mediated inhibition of [^{3}H]noradrenaline release from rat hippocampus is reduced by 4-aminopyridine,but that caused by an adenosine analogue or omega-conotoxin is not. *Acta Physiol. Scand.,* **136**, 347-353.

[88] Jolas, T., Aghajanian, G. K. (1997) Opioids supress spontaneous and NMDA-induced inhibitory postsynaptic currents in the dorsal raphe nucleus of the rat *in vitro*. *Brain Res.,* **755**, 229-245.

[89] Judge, S., Gartside, S. E. (2006) Firing of 5-HT neurones in the dorsal and median raphe nucleus *in vitro* shows differential alpha1-adrenoceptor and 5-HT_{1A} receptor modulation. *Neurochem. Internat.,* **48**, 100-107.

[90] Juranyi, Zs., Harsing, L. G. Jr. (2004) Brain slice chambers designed for *in vitro* experiments with nervous tissue, in: Torok, T. L., Klebovich, I. Eds, Monoamine Oxidase Inhibitors Budapest, Medicina, pp. 281-308.

[91] Kalen, P., Wiklund, L. (1989) Projections from the medial septum and diagonal band of Broca to the dorsal and central superior raphe nuclei: a non-cholinergic pathway. *Exp. Brain Res.,* **75**, 401-416.

[92] Kapadia, S. E., De Lanerolle, N. C., Lamotte, C. C. (1985) Immunocytochemical and electron microscopic study of serotonin neuronal organization in the dorsal raphe nucleus of the monkey. *Neuroscience,* **16**, 729-746.

[93] Kerwin, R. W., Pycock, C. J. (1979) The effect of some putative neurotransmitters on the release of 5-hydroxytryptamine and gamma-aminobutyric acid from slices of the rat midbrain raphe area. *Neuroscience,* **4**, 1359-1365.

[94] Kidd, E. J., Garratt J, C., Marsden, C. A. (1991) Effects of repeated treatment with 1-(2,3-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) on the autoregulatory control of dorsal raphe 5-HT neuronal firing and cortical 5-HT release. *Eur. J. Pharmacol.,* **200**, 131-139.

[95] Kita, H. (1996) Glutamatergic and GABAergic postsynaptic responses of striatal spiny neurons to intrastriatal and cortical stimulation recorded in slice preparations. *Neuroscience,* **70**, 925-940.

[96] Klimek, V., Roberson, G., Stockmeier, C. A., Ordway, G. A. (2003) Serotonin transporter and MAO-B levels in monoamine nuclei of the human brainstem are normal in major depression. *J. Psychiat. Res.,* **387,** 387-397.

[97] Knoll, J. (1993) The pharmacological basis of the therapeutic effect of (-)-deprenyl in age related neurological diseases, in: Szelenyi, I. Ed, Inhibitors of Monoamine Oxidase Basel, Birkhauser Verlag, pp. 145-168.

[98] Krogsgaard-Larsen, P., Frolund, B., Jorgensen, F. S., Schousboe, A. (1994) $GABA_A$ receptor agonists,partial agonists,and antagonists. Design and therapeutic prospects. *J. Med. Chem.,* **37**, 2489-2505.

[99] Langer, S. Z., Moret, C. (1982) Citalopram antagonizes the stimulation by LSD of presynaptic inhibitory 5-HT autoreceptors in the rat hypothalamus. *J. Pharm. Exp. Ther.,* **222**, 220-226.

[100] Lewitt, P., Pintar, J. E., Breakefield, X. O. (1982) Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Proc. Natl. Acad. Sci. USA,* **79**, 6385-6389.

[101] Liu, R. J., Jolas, T., Aghajanian, G. K. (1999) 5-HT, *via* 5-HT_2 receptors, induces an increase in spontaneous IPSCs in 5-HT cells of the dorsal raphe nucleus (DRN). *Soc. Neurosci. Abstr.,* **174**, 9.

[102] Lovell, P. J., Bromidge, S. M., Dabbs, S., Duckworth, D. M., Forbes, I. T., Jennings, A. J., King, F. D., Middlemniss, D. N., Rahman, S. K., Saunders, D. V., Collin, L. L., Hagan, J. J., Riley, G. J., Thomas, D. R. (2000) A novel, potent, and selective 5-HT_7 antagonist: (R)-3-(2-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol (SB-269970). *J. Med. Chem.,* **43**, 342-345.

[103] Luppi, P. H., Petit, J. M., Rampon, C., Jouvet, M. (1998) Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. *Neuroscience,* **82**, 443-468.

[104] Magoul, R., Onteniente, B., Oblin, A., Calas, A. (1986) Inter- and intracellular relationship of substance P-containing neurons with serotonin and GABA in the dorsal raphe nucleus: combination of autoradiographic and immunocytochemical tchniques. *J. Histochem. Cytochem.,* **34**, 735-742.

[105] Mannoury, l. C. C., Hanoun, N., Melfert, M., Hen, R., Lesch, K. -P., Hamon, M., Lanfumey, L. (2004) $GABA_B$ receptors in 5-HT transporter and 5-HT_{1A} receptor-knock-out mice: further evidence of a transduction pathway share with 5-HT_{1A} receptors. *J. Neurochem.,* **89**, 886-896.

[106] Martin-Ruiz, M., Puig, M. V., Celada, P., Shapiro, D. A., Roth, B. L., Mengod, G., Artigas, F. (2001) Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. *J. Neurosci.,* **21**, 9856-9866.

[107] Martin, K. F., Hannon, S., Phillips, I., Heal, D. J. (1992) Opposing roles for 5-HT_{1B} and 5-HT_3 receptors in the control of 5-HT release in rat hippocampus *in vivo*. *Br. J. Pharmacol.,* **106**, 139-142.

[108] Mefford, N. N. (1981) Application of high performance liquid chromatography with electrochemical detection to the neurochemical analysis: measurement of catecholamines, 5-HT and metabolites in rat brain. *J. Neurosci. Methods,* **3**, 207-224.

[109] Middlemiss, D. N., Fozard, J. R. (1983) 8-Hydroxy-2-(di-n-propylamino) tetralin discriminates between subtypes of the 5-HT_1 recognition site. *Eur. J. Pharmacol.,* **90**, 151-153.

[110] Middlemiss, D. N. (1984) Stereoselective blockade at [^{3}H]5-HT binding sites and at the 5-HT autoreceptor by (-)propranolol. *Eur. J. Pharmacol.,* **101**, 289-293.

[111] Moore, R. Y., Card, J. P. (1984) Noradrenaline-containing neuron systems, in: Bjorklund, A., Hokfelt, T. Eds, Handbook of Chemical Neuroanatomy. Classical Neurotransmitters in the CNS, Vol. 2 Edition. Amsterdam, Elsevier, pp. 123-156.

[112] Nanopoulos, D., Belin, M. F., Maitre, M., Vincendon, G., Pujol, J. F. (1982) Immunocytochemical evidence for the existence of GABAergic neurons in the nucleus raphe dorsalis. Possible existence of neurons containing serotonin and GABA. *Brain Res.,* **232**, 375-389.

[113] Neale, R. F., Fallon, S. L., Royar, W. C., Wasley, J. W. F., Martin, L. L., Stone, G. A., Glaester, B. S., Sinton, C. M., Williams, M. (1987) Biochemical and pharmacological characterization of CGS-12066A, a selective 5-HT_{1B} agonist. *Eur. J. Pharmacol.,* **136**, 1-9.

[114] Nishikawa, T., Scatton, B. (1985) Inhibitory influence of GABA on central serotonergic transmission. Involvement of the habenulo-raphe pathways in the GABAergic inhibition of ascending cerebral serotonergic neurons. *Bra,* **331**, 81-90.

[115] O'Connor, J. J., Kruk, Z. L. (1992) Pharmacological characteristics of 5-hydroxytryptamine autoreceptors in rat brain slices incorporating the dorsal raphe or the suprachiasmatic nucleus. *Br. J. Pharmacol.,* **106**, 524-532.

[116] O'Hearn, E., Molliver, M. E. (1984) Organization of raphe-cortical projections in the rat: a quantitative retrograde study. *Brain Res. Bulletin,* **13**, 709-726.

[117] Palacios, J. M., Pazos, A., Hoyer, D. (1987) Characterization and mapping of 5-HT_{1A} sites in the brain of animals and man, in: Dourish, C. T., Ahlenins, S., Hutson, P. H. Eds, Brain 5-HT_{1A} Receptors Chichester, Ellis Horwood, pp. 67-81.

[118] Pan, Z. Z., Colmers, W. F., Williams, J. T. (1989) 5-HT-mediated synaptic potentials in the dorsal raphe nucleus: interactions with excitatory amino acid and GABA neurotransmission. *J. Neurophysiol.,* **62**, 481-486.

[119] Pan, Z. Z., Williams, J. T. (1989) GABA- and glutamate-mediated synaptic potentials in rat dorsal raphe neurons *in vitro*. *J. Neurophysiol.,* **61**, 719-726.

[120] Pan, Z. Z., Grudt, T. J., Williams, J. T. (1994) Alpha 1-adrenoceptors in rat dorsal raphe neurons: egulation of two potassium conductances. *J. Physiol.,* **478**, 437-447.

[121] Parent, A., Descarries, L., Beaudet, A. (1981) Organization of ascending serotonin systems in the adult rat. A radioautographic study after intraventricular administration of [^{3}H]5-hydroxytryptamine. *Neuroscience,* **6**, 115-138.

[122] Pelayo, F., Dubocovich, M. L., Langer, S. Z. (1980) Inhibition of neural uptake reduces the presynaptic effects of clonidine but not of -methyl-noradrenaline on the stimulation-evoked release of [^{3}H]-noradrenaline from rat occipital cortex slices. *Eur. J. Pharmacol.,* **64**, 143-155.

[123] Penington, N. J., Kelly, J. S. (1990) Serotonin receptor activation reduces calcium receptor activation reduces calcium current in an acutely dissociated adult central neuron. *Neuron,* **4**, 751-758.

[124] Peyron, C., Luppi, P. H., Fort, P., Rampon, C., Jouvet, M. (1996) Lower brainstem catecholamine afferents to the rat dorsal raphe nucleus. *J. Comp. Neurol.,* **364**, 402-413.

[125] Pineyro, G., Blier, P. (1996) Regulation of 5-hydroxytryptamine release from rat midbrain raphe nuclei by 5-hydroxytryptamine1D receptors: Effect of tetrodotoxin, G protein inactivation and long-term antidepressant administration. *J. Pharm. Exp. Ther.,* **276**, 697-707.

[126] Pineyro, G., Blier, P. (1999) Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacol. Rev.,* **51**, 533-591.

[127] Pobbe, R. L. H., Zangrossi, H., Jr. (2005) 5-HT_{1A} and 5-HT_{2A} receptors in the rat dorsal periaqueductal gray mediate the antipanic-like effect induced by the stimulation of serotonergic neurons in the dorsal raphe nucleus. *Psychopharmacology*, **183,** 314-321.

[128] Price, G. W., Burton, M. J., Collin, L. J., Duckworth, M., Gaster, L., Gothert, M., Jones, B. J., Roberts, C., Watson, J. M., Middlemniss, D. N. (1997) SB-216641 and BRL-15572-compounds to pharmacologically discriminate h5-HT_{1B} and h5-HT_{1D} receptors. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **356**, 312-320.

[129] Pudovkina, O. L., Cremers, T. I., Westerink, B. H. (2003) Regulation of the release of serotonin in the dorsal raphe nucleus by alpha1 and alpha2 adrenoceptors. *Synapse*, **50**, 77-82.

[130] Rasmussen, K., McCreary, A. C., Shanks, E. A. (2004) Attenuation of the effects of fluoxetine on serotonergic neural activity by pindolol in rats. *Neurosci. Letters,* **355**, 1-4.

[131] Roberts, C., Watson, J., Burton, M., Price, G. W., Jones, B. J. (1996) Functional characterization of the 5-HT terminal autoreceptor in the guinea-pig brain cortex. *Br. J. Pharmacol.,* **117**, 384-388.

[132] Roberts, C., Allen, L., Langmead, C. J., Hagan, J. J., Middlemniss, D. N., Price, G. W. (2001) The effect of SB-269970,a selective 5-HT_7 receptor antagonist,on 5-HT release from serotonergic terminals and cell bodies. *Br. J. Pharmacol.,* **132**, 1574-1580.

[133] Rosin, D. L., Zeng, D., Stornetta, R. L., Norton, F. R., Riley, T., Okusa, M. D., Guyenet, P. G., Lynch, K. R. (1993) Immunohistochemical localization of alpha2A-adrenergic receptors in catecholaminergic and other brainstem neurons in the rat. *Neuroscience*, **56**, 139-155.

[134] Rowley, H. L., Martin, K. F., Marsden, C. (1995) A Determination of *in vivo* amino acid neurotransmitters by high performance liquid chromatography with o-phthalaldehyde-sulphite derivatisation. *J. Neurosci. Methods,* **57**, 93-99.

[135] Ruat, M., Traiffort, E., Luers, R., Tardivel-Lacombe, J., Diaz, J., Arrang, J. M., Schwartz, J. C. (1993) Molecular cloning, characterization,and localization of a high-affinity serotonin receptor (5-HT_7) activating cAMP formation. *Proc. Natl. Acad. Sci. USA,* **90**, 8547-8551.

[136] Rudy, B. (1988) Diversity and ubiquity of K channels. *Neuroscience,* **25**, 729-749.

[137] Saavedra, J. M., Grobecker, H., Zivin, J. (1976) Cathecholamines in the raphe nuclei of the rat. *Brain Res.,* **114** 337-345.

[138] Sakai, K., Salvert, D., Touret, M., Jouvet, M. (1977) Afferent connections of the nucleus raphe dorsalis in the cat as visualized by the horseradish peroxidase technique. *Brain Res.,* **137**, 11-35.

[139] Sakai, K., Crochet, S. (2001) Differentiation of presumed serotonergic dorsal raphe neurons in relation to behavior and wake-sleep states. *Neuroscience,* **104**, 1141-1155.

[140] Saura, J., Kettler, R., Da Prada, M., Richards, J. G. (1992) Quantitative enzyme radioautography with 3H-Ro 41-1049 and 3H-Ro 19-6327 *in vitro*: localization and abundance of MAO-A and MAO-B in rat CNS,peripheral organs,and human brain. *J. Neurosci.,* **12**, 1977-1999.

[141] Scatton, B., Serrano, A., Rivot, J. P., Nishikawa, T. (1984) GABAergic inhibitory influence on striatal serotonergic transmission exerted in the dorsal raphe as revealed by *in vivo* voltametry. *Brain Res.,* **305**, 343-352.

[142] Schlicker, E., Gothert, M., Clausing, R. (1982) Acute and chronic changes of noradrenergic transmission do not affect the alpha-adrenoceptor-mediated inhibition of [^{3}H]serotonin release in the cerebral cortex. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **320**, 38-44.

[143] Schlicker, E., Fink, K., Moldering, G. J., Prince, G. W., Duckworth, D. M., Gaster, L., Middlemniss, D. N., Zentner, J., Likungu, J., Gothert, M. (1997) Effects of selective h5-HT_{1B} (SB-216641) and h5-HT_{1D} (BRL-15572) receptor ligands on guinea-pig and human auto- and heteroreceptors. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **356**, 321-327.

[144] Schoeffter, P., Hoyer, D. (1989) 5-Hydroxytryptamine 5-HT_{1B} and 5-HT_{1D} receptors mediating inhibition of adenylate cyclase activity. Pharmacological comparison with special reference to the effects of yohimbine,rauwolscine and some beta-adrenoceptor antagonists. *Naunyn-Schmiedeberg's Arc. Pharmacol.,* **340**, 285-292.

[145] Sershen, H., Hashim, A., Lajtha, A. (1995) The effect of ibogaine on kappa-opioid and 5-HT_3-induced changes in stimulation-evoked dopamine release *in vitro* from striatum of C57/BL/6By mice. *Brain Res. Bulletin,* **36**, 587-591.

[146] Sesack, S. R., Deutch, A. Y., Roth, R. H., Bunney, B. S. (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J. Comp. Neurol.,* **290**, 213-242.

[147] Shen, R. Y., Andrade, R. (1998) 5-Hydroxytryptamine2 receptor facilitates GABAergic neurotransmission in rat hippocampus. *J. Pharm. Exp. Ther.,* **285**, 805-812.

[148] Smith,D. A., Gallager, D. W. (1989) Electrophysiological and biochemical characterization of the development of alpha-1 adren-

ergic and 5-HT_1 receptors associated with dorsal raphe neurons. *Dev. Brain Res.,* **46**, 173.

[149] Spouse, J. S., Aghajanian, G. K. (1987) Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonists. *Synapse,* **1**, 3-9.

[150] Spouse, J., Reynolds, L., Rollema, H. (1997) Do 5-$HT_{1B/1D}$ autoreceptors modulate dorsal raphe cell firing? *In vivo* electrophysiological studies in guinea-pigs with GR127935. *Neuropharmacology,* **36**, 559-567.

[151] Stamford, J. A., Davidson, C., McLaughlin, D. P., Hopwood, S. E. (2000) Control of dorsal raphe 5-HT function by multiple 5-HT_1 autoreceptors: parallel purposes or pointless plurality? *Trends Neurosci.,* **23**, 459-465.

[152] Starke, K. (1987) Presynaptic alpha-adrenoceptors. *Rev. Physiol. Biochem. Pharmac.,* **107**, 73-146.

[153] Starley, S. J., Skingle, M. (1994) 5-HT_{1D} as well as 5-HT_{1A} autoreceptors modulate 5-HT release in the guinea-pig dorsal raphe nucleus. *Neuropharmacology,* **33**, 395-402.

[154] Steinbusch, H. W. M. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-Cell bodies and terminals. *Neuroscience,* **6**, 557-618.

[155] Stern, W. C., Johnson, A., Bronzino, J. D., Morgane, J. P. (1981) Neuropharmacology of the afferent projection from the lateral habenula and substantia nigra to the anterior raphe in the rat. *Neuropharmacology,* **20**, 979-989.

[156] Stowe, R. L., Barnes, N. M. (1998) Selective labelling of 5-HT_7 recognition sites in rat brain using [^{3}H]-5-carboxamidotryptamine. *Neuropharmacology,* **37**, 1611-1619.

[157] Talley, E. M., Rosin, D. L., Lee, A., Guyenet, P. G., Lynch, K. R. (1996) Distribution of alpha2A-adrenergic receptor-like immunoreactivity in the rat central nervous system. *J. Comp. Neurol.,* **372**, 111-134.

[158] Tao, R., Ma, Z., Auerbach, S. B. (1996) Differential regulation of 5-hydroxytryptamine release by $GABA_A$ and $GABA_B$ receptors in midbrain raphe nuclei and forebrain of rats. *Br. J. Pharmacol.,* **119**, 1375-1384.

[159] Tao, R., Auerbach, S. B. (2002) GABAergic and glutamatergic afferents in the dorsal raphe nucleus mediate morphine-induced increases in serotonin efflux in the rat central nervous system. *J. Pharm. Exp. Ther.,* **303**, 704-710.

[160] Thomas, D. R., Gittins, S. A., Collin, L. L., Middlemniss, D. N., Riley, G., Hagan, J., Gloger, I., Ellis, C. E., Forbes, I. T., Brown, A. M. (1998) Functional characterization of the human cloned 5-HT_7 receptor (long form); antagonists profile of SB-258719. *Br. J. Pharmacol.,* **124**, 1300-1306.

[161] To, Z. P., Bonhaus, D. W., Eglen, R. M., Jakeman, L. B. (1995) Characterization and distribution of putative 5-ht_7 receptors in guinea-pig brain. *Br. J. Pharmacol.,* **115**, 107-116.

[162] Van der Kooy, D., Hattori, T. (1980) Dorsal raphe cells with collateral projections to the caudate-putamen and substantia nigra: a fluorescent retrograde double labeling study in the rat. *Brain Res.,* **186**, 1-7.

[163] Vandermaelen, C. P., Aghajanian, G. K. (1983) Electrophysiological and pharmacological characterization of serotonin dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. *Brain Res.,* **289**, 109-119.

[164] Vanhoenacker, P., Haegemann, G., Leysen, J. E. (2000) 5-HT_7 receptors: current knowledge and future prospects. *Trends Pharm. Sci.,* **21**, 70-77.

[165] Varga, V., Szekely, A. D., Csillag, A., Sharp, T., Hajos, M. (2001) Evidence for a role of GABA interneurones in the cortical modulation of midbrain 5-hydroxytryptamine neurones. *Neuroscience,* **106**, 783-792.

[166] Varga, V., Kocsis, B., Sharp, T. (2003) Electrophysiological evidence for convergence of inputs from the medial prefrontal cortex and lateral habenula on single neurons in the dorsal raphe nucleus. *Eur. J. Neurosci.,* **17**, 280-286.

[167] Verge, D., Daval, G., Patey, A., Gozlan, H., El Mestikawy, S., Hamon, M. (1985) Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT_{1A} subtype. *Eur. J. Pharmacol.,* **113**, 70-77.

[168] Vizi, E. S., Harsing, L. G. Jr., Zsilla, G. (1981) Evidence of the modulatory role of serotonin in acetylcholine release from striatal interneurons. *Brain Res.,* **212**, 463-464.

[169] Vizi, E. S., Somogyi, G. T., Harsing, L. G., Zimanyi, I. (1985) External Ca-independent release of norepinephrine by sympathomimietics and its role in negative feedback modulation. *Proc. Natl. Acad. Sci. USA,* **82**, 89-99.

[170] Vizi, E. S. (2000) Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the CNS. *Pharmacol. Reviews,* **55**, 8775-8779.

[171] Voigt, M. M., Laurie, D. J., Seeburgh, P. H., Bach, A. (1991) Molecular cloning and characterization of a rat brain cDNA encoding a 5-hydroxytryptamine1B receptor. *EMBO J.,* **10**, 63-89.

[172] Waeber, C., Moskowitz, M. A. (1995) Autoradiographic visualisation of [^{3}H]-5-carboxamidotryptamine binding sites in guinea-pig and rat brain. *Eur. J. Pharmacol.,* **283**, 4017-4023.

[173] Wang, R. Y., Aghajanian, G. K. (1977) Physiological evidence for habenula as major link between forebrain and midbrain raphe. *Science,* **197**, 31-46.

[174] Wang, Q. P., Ochiaia, P. G., Nakai, Y. (1992) GABAergic innervation of serotonergic neurons in the dorsal raphe nucleus of the rat studied by electron microscopy double immunostaining. *Brain Res. Bulletin,* **29**, 89-91.

[175] Wiklund, L., Bjorklund, A. (1980) Mechanism of regrowth in the bulbospinal 5-HT system following 5,6-dihydroxytryptamine induced axotomy. II. Fluorescence histochemical observations. *Brain Res.,* **191**, 943-948.

[176] Wiklund, L., Leger, L., Persson, M. (1981) Monoamine cell distribution in the cat brain stem. A fluorescence histochemical study with quantification of indolaminergic and locus coeruleus cell groups. *J. Comp. Neurol.,* **203**, 613-647.

[177] Wright, D. E., Seroogy, K. B., Lundgren, K. H., Davis, B. M., Jennes, L. (1995) Comparative location of serotonin1A,1C and 2 receptor subtype mRNAs in rat brain. *J. Comp. Neurol.,* **351**, 357-373.

[178] Yakel, J. L., Jackson, M. B. (1988) 5-HT_3 receptors mediate rapid responses in cultured hippocampal and clonal cell line. *Neuron,* **1**, 615-621.

[179] Yang, H. Y. T., Neff, N. H. (1973) Beta-Phenethylamine: A specific substrate for type B monoamine oxidase of brain. *J. Pharm. Exp. Ther.,* **187**, 365-371.

[180] Zifa, E., Fillion, G. (1992) 5-Hydroxytryptamine receptors. *Pharmacol. Rev.,* **44**, 401-458.

[181] Zsilla, G., Barbaccia, M. L., Gandolfi, O., Knoll, J., Costa, E. (1983) (-)Deprenyl,a selective MAO "B" inhibitor increases [^{3}H]imipramine binding and decreases beta-adrenergic receptor function. *Eur. J. Pharmacol.,* **89**, 111-117.

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