5-HT\textsubscript{1A} Receptor, an Old Target for New Therapeutic Agents

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Abstract: The serotonin receptor subtype 5-HT\textsubscript{1A} was one of the first serotonin receptor subtypes pharmacologically characterized. Over the last twenty years the 5-HT\textsubscript{1A} receptor has been the object of intense research efforts as witnessed by the 5-HT\textsubscript{1A} acting drugs marketed as anxiolytics. In recent years, several new chemical entities targeting the 5-HT\textsubscript{1A} receptor (alone or in combination with other molecular targets) have been proposed for novel therapeutic indications (neuroprotection, cognitive impairment, Parkinson Disease and related disorders, pain treatment). The present review will focus on those 5-HT\textsubscript{1A} receptor agents that entered preclinical trials starting from 2000.

INTRODUCTION

Serotonin (5-HT) exerts its diverse actions by binding to distinct cell surface receptors which have been classified into many groups on the basis of their pharmacological responses to specific ligands, sequence similarities at the gene and amino acid levels, gene organization, and second messenger coupling pathways. The development of ligands with enhanced specificity, the molecular cloning of several of these receptors, and subsequent heterologous expression have unambiguously confirmed the existence of at least 14 subtypes of serotonin receptors [1]. Most of the serotonin receptors, except the 5-HT\textsubscript{3}, belong to the large family of seven transmembrane domain G-protein coupled receptors [2]. The 5-HT\textsubscript{1A} receptor is an important member of this large family of receptors. The 5-HT\textsubscript{1A} receptor has been the most extensively studied of the serotonin receptors for a number of reasons. One of the main reasons resides on the early availability of the selective agonist 8-hydroxy-2-(di-N-propylamino)tetratin (8-OH-DPAT) [3, 4] that has allowed extensive biochemical, physiological, and pharmacological characterization of the receptor. The 5-HT\textsubscript{1A} receptor was the first among all the serotonin receptors to be cloned and sequenced [5-7].

According to the data collected by the PubMed [8] starting from 1990, the number of publications per year containing the term “5-HT\textsubscript{1A}” into the title and the abstract revealed an interesting trend (Fig. 1). The number of papers increased constantly until 1996, with a peak of about 400 papers. In 2000, the number of publications nearly halved, whereas in recent years the number of papers increased, witnessing a renewed interest.

In 1986, buspirone was introduced by Bristol-Myers Squibb under the brand name BuSpar as a medication to treat patients suffering from generalized anxiety disorder (GAD). Clinical trials demonstrated that buspirone was effective in the treatment of anxiety with efficacy and dosage comparable to diazepam or chlorazepate. Moreover, due to its different site of action it lacked of the anticonvulsant, sedative, and muscle-relaxant properties associated with other anxiolytics. Also, it lacked liability to produce physical dependence or to significantly interact with central nervous system depressants such as ethanol. With its low incidence of sedation, buspirone was presented as a useful addition to the treatments available for GAD and it was seen as a promising therapeutic agent in patients in whom daytime alertness was particularly important [9, 10]. Buspirone also appeared to have efficacy in major depressive disorders, suggesting that modulation of 5-HT\textsubscript{1A} receptor function might have clinical antidepressant properties [11]. However, buspirone is poorly selective and this originates several unwanted side-effects including dizziness, nausea, insomnia, headaches, nervousness, and lightheadness. Moreover, the main metabolite of buspirone, 1-(2-pyrimidyl)piperazine, displays a variety of pharmacological actions.

The development of buspirone opened the avenue for the search of new anxiolytic drugs acting at 5-HT\textsubscript{1A} receptors. In recent years a number of 5-HT\textsubscript{1A} agonists entered Phase II clinical trials as anxiolytics (S 15535, alnespirone, MN-305, lesopitron, gepirone, flesinoxan; Fig. 2). On the other hand, the possible involvement of 5-HT\textsubscript{1A} receptor function in depression therapy originated several compounds acting at various molecular targets, including 5-HT\textsubscript{1A} receptors. For...
instance, flibanserin, DU-125530, vilazodone, robalzotan, and OPC-14523 (Fig. 3) have reached late stage clinical development.

Early studies on the pharmacological profile of atypical antipsychotics revealed an interesting role for 5-HT1A receptor component. Activation of 5-HT1A receptor demonstrated to reduce motor side-effects induced by activation of dopamine D2 receptors. On such basis, several mixed D2/5-HT2A/5-HT1A agents have been studied over the years. Perospirone and ziprasidone are two valuable examples of this multitarget strategy that have reached the market in 2001 and 2002, respectively. Among many other agents with a similar pharmacological profile, SLV313 and bifeprunox (Fig. 4) entered Phase I and Phase III clinical trials, respectively.

Besides these well-established therapeutic areas, other, yet interesting, therapeutical perspectives have emerged from the careful evaluation of the effects elicited by some 5-HT1A receptors agents. The present review will focus on the therapeutic potential of 5-HT1A agents that entered preclinical trials starting from 2000 and will highlight their key pharmacological features.

**NEUROPROTECTION**

One of the fundamental mechanisms in the process of ischemic cell death is ionic imbalance and excitotoxicity mediated by glutamate release. The neuroanatomical distribution of 5-HT1A receptors, coupled with hyperpolarization of cell membrane and glutamate release inhibition elicited by 5-HT1A receptor agonists, suggested a neuroprotective role on the brain in cerebral ischemic conditions.

5-HT1A receptors are highly expressed postsynaptically in areas of the brain that are most sensitive to neuronal damage following ischemic stroke or brain trauma, such as hippocampus (CA1 and CA3 sectors and dentate gyrus), cerebral cortex and basal forebrain nuclei [12-14]. The 5-HT1A receptors exert an inhibitory effect on the firing activity of neurons in these brain areas. Stimulation of the 5-HT1A subtype has been shown to induce neuronal hyperpolarization, most likely mediated by activation of G-protein coupled K+ channels, and consequent inhibition of neuronal activity [13, 15-17]. In addition, 5-HT1A receptor agonists produce voltage-independent blockade of N-, P-/Q-, and T-type voltage-dependent Ca2+ channels via G-protein-mediated signaling mechanisms or via a membrane-delimited pathway, respectively [18]. Furthermore, 5-HT1A signaling inhibits L-type voltage-dependent Ca2+ channel through a G-protein-mediated diffusible cytosolic messenger,19 and by this way abrogate Ca2+ entry into nerve cells [17]. 5-HT1A receptor agonists can reduce glutamate release from nerve terminals [17, 20], which is most likely mediated through activation of presynaptic 5-HT1A receptors located on glutamatergic terminals [21, 22].

The potential of 5-HT1A receptor agonists for the treatment of ischemic brain disorders was revealed by several studies starting from 1990. In particular, 8-OH-DPAT, buspirone, gepirone, ipsapirone, Bay R 1531 were tested in rat and mouse after induction of focal cerebral ischemia and demonstrated to be efficacious in decreasing the area of cerebral damage [23]. Also urapidil and CM 57493 demonstrated to reduce dose dependently the infarct size in rodent models of focal and global ischemia [24]. On the other hand, conflicting results were obtained in gerbil models. Bode-Gruel and coworkers [25] found that the partial agonist ipsapirone and the full agonist Bay R 1531 displayed neuroprotectant activity, whereas gepirone and 8-OH-DPAT were ineffective. Piera et al.26 studied 8-OH-DPAT, buspirone and flesinoxan on neuronal degeneration induced by transient global ischemia.
med its ability to reduce neuronal damage, whereas buspirone and flesinoxan failed to protect against neuronal damage.

All the above studies clearly pointed out the potential of 5-HT1A receptor agonists as neuroprotectants. However, the recovery from ischemic damage elicited by the above agonists was quite limited at doses not suitable for therapeutic use. For instance, after permanent middle cerebral artery occlusion (p-MCA-O), CM 57493 reduced the cortical infarct volume by 30% at 1-5 mg/kg dose and ipsapirone produced a 60% reduction at 30 mg/kg.

Starting from these observations, it was emphasized that, besides selectivity over other receptor systems, the level of affinity and intrinsic activity played a pivotal role in antischemic effect. It was hypothesized that the therapeutic spectrum of a 5-HT1A receptor agonist could be optimized by increasing the apparent intrinsic activity at pre- and post synaptic 5-HT1A receptor population. With this respect, De Vry observed that the so-called full agonist 8-OH-DPAT appeared not to have the same intrinsic activity as 5-HT itself at those receptors responsible for the membrane hyperpolarizing effects of 5-HT in the CA1 sector of hippocampus [13]. These findings and considerations stimulated the search for more selective 5-HT1A receptor ligands endowed with full agonist properties, demonstrated in a wide array of functional assays.

Repinotan (formerly known as BAY x 3407; Fig. 5) represents a potent and relatively selective 5-HT1A agonist which fulfills the above mentioned requirements. The pharmacological profile of this agent as well as its neuroprotective properties in several models of ischemia and brain trauma have been extensively reviewed [27-29]. Here is reported a brief summary of repinotan pharmacological profile.

Repinotan binds with high affinity at 5-HT1A receptors from various tissues, including calf hippocampus (K_i = 0.19 nM), rat cortex (K_i = 0.24 nM), rat hippocampus (K_i = 0.58 nM), human cortex (K_i = 0.25 nM), and recombinant 5-HT1A receptors (K_i = 0.4 nM). Repinotan binds also with relatively high to moderate affinity to the other monoaminergic receptors: α1 and α2 adrenergic (K_i = 6 and 7 nM, respectively); 5-HT7 and 5-HT1D (K_i = 7 and 36 nM, respectively); dopamine D2 and D4 (48 and 91 nM, respectively). In more than 50 other receptor binding assays, weak affinity was only detected for 5-HT2C receptors (K_i = 310 nM) and σ binding sites (K_i = 176 nM). The binding profile of repinotan is considered to be relatively selective because at least one order of magnitude differentiates its binding to the 5-HT1A receptor from its binding to other receptors.

The intrinsic activity of repinotan has been assessed by using several models. In the forskolin-stimulated adenylate cyclase assay in rat hippocampal tissue, a model of

**Fig. (3).** 5-HT1A receptor agonists as potential antidepressants interacting also with other molecular targets.

**Fig. (4).** Potential antipsychotics with 5-HT1A receptor agonist activity.
postsynaptic 5-HT1A receptor function, repinotan was a potent 5-HT1A receptor full agonist (IC50= 3.5 nM), considerably more potent than the standard 5-HT1A receptor full agonist 8-OH-DPAT (IC50= 24 nM). Its effect was completely blocked by the selective 5-HT1A receptor antagonist WAY-100635 (Fig. 6). The agonistic properties of repinotan were confirmed also at presynaptic level in an in vitro electrophysiological assay. In fact, in rat brain slice preparation containing dorsal raphe nucleus (DRN), repinotan induced a long lasting inhibition of neuronal firing at concentration of 1 nM. Also in vivo, repinotan has been characterized as a 5-HT1A receptor agonist. Repinotan suppressed 5-HT neuronal firing in the DRN after intravenous application in anesthetized rat. In another in vivo model, rats trained to discriminate 8-OH-DPAT from vehicle, potent and complete generalization was obtained after administration of repinotan.

The agonistic properties at pre- and postsynaptic 5-HT1A receptors of repinotan were also evidenced in vivo. In fact, repinotan induced potent and dose-dependent effects in behavioral models highly sensitive to 5-HT1A receptor agonism (rat forced swimming test, shock-induced ultrasonic vocalization). Finally, repinotan induced, at higher doses, different components of the 5-HT-dependent behavioural syndrome in rats, including reciprocal forepaw threading, Straub tail, increase in locomotor activity, hindlimb abduction and flat body posture, and hypothermia. Repinotan has been tested in various models that mimic different aspects of human brain injury: permanent focal cerebral ischemia model involving pMCA-O; reperfusion injury model by transient middle artery occlusion (tMCA-O); traumatic brain injury (TBI) model involving induction of acute subdural hematoma (aSDH). Also, two different administration techniques have been used: i.e. continuous infusion and trible bolus injection (Table 1). Repinotan demonstrated pronounced neuroprotectant properties. Moreover, repinotan showed a therapeutic time-window of at least 5 h from the ischemic event in the same animal models (Table 2).

In 2004 Bayer HealthCare [30] reported the ending of development program for repinotan (Branosyn), because it did not fulfill expectations after completion of Phase IIb clinical trial. However, no scientific report about this point appeared into the literature.

The japanese company Daiichi Asubio Pharma has disclosed in recent years the selective 5-HT1A receptor agonist piclozotan (SUN N4057, Fig. 5). This compound showed remarkable neuroprotective activity in a tMCA-O model. In an early report, piclozotan was reported as structurally derived from the prototypical “long-chain” arylpiperazine derivative buspirone [31]. Piclozotan demonstrated improved selectivity over dopamine D2 receptor as compared to buspirone (piclozotan: 5-HT1A IC50= 0.47 nM, D2 IC50 = 84 nM; buspirone: 5-HT1A IC50= 11.0 nM, D2 IC50 = 55 nM). Moreover, piclozotan showed binding affinities to other receptors as follows: 5-HT2A: IC50>1000 nM; dopamine D1: IC50 >1000 nM; α1 adrenergic: IC50 = 128 nM; α2 adrenergic: IC50 = 228 nM. Piclozotan demonstrated to be a postsynaptic 5-HT1A receptor agonist because it inhibited
forskolin-stimulated adenylate cyclase activity in plasma membrane prepared from the rat hippocampus (IC
50 = 2.67 nM). On the basis of such profile, the authors investigated *in vivo* the neuroprotective effect of piclozotan in a rat model of transient focal ischemia. The compound was subcutaneously administered immediately after t-MCA-O in rats. The neuronal damage was quantitated from the level of peripheral type benzodiazepine binding sites (PTBBS) in ipsilateral cortical and striatal homogenates, 10 days after recirculation. Piclozotan and buspirone (1 mg/kg s.c.) reduced the increase in PTBBS levels of 63% and 53%, respectively. The hypothermic effect of piclozotan was not found to be related to the neuroprotective effect [32]. Piclozotan is currently in Phase IIb clinical trials for treatment of ischemic stroke [33]. Unfortunately, no other data on intrinsic activity at 5-HT 1A receptor of piclozotan both in vitro and *in vivo* are available. Therefore a peer-to-peer comparison of repinotan and piclozotan cannot be done and a general profile for 5-HT 1A agonists with anti ischemic properties cannot be drawn.

**COGNITIVE IMPAIRMENT**

Alzheimer’s disease is a neurodegenerative disorder characterized by multiple deficits in neurotransmitters function. Numerous studies have documented a reduction in glutamate release in several brain areas (neocortex, hippocampus, enthorinal cortex). Glutamate plays a pivotal role in cognition, learning and memory processes. In particular, the activation of glutamatergic neurotransmission facilitates memory, whereas transmission blockade impairs learning and memory. On the other hand, the serotonergic system results to be hyperactive as a result of the enhanced turnover of serotonin which would reduce the neuronal firing through stimulation of 5-HT 1A receptors.

It has been demonstrated that 5-HT 1A receptors located on presynaptic glutamatergic neurons could inhibit both excitatory neurotransmission and release of glutamate in vitro. Therefore, it has been suggested that 5-HT 1A receptor antagonists could have a facilitatory effect on glutamatergic transmission by blocking the hyperpolarization and changes in Ca 2+ flux induced by inhibitory serotonergic tone [34]. Finally, it has been speculated that 5-HT 1A receptor antagonists may decrease the formation of β-amyloid peptide which has been clearly linked to neuronal plaques that are the pathological hallmarks of the disease [35].

Starting from 1993, several selective 5-HT 1A receptor antagonists has been developed, including WAY-100135,
WAY-100635, WAY-405, NAD-299, and LY-426965. Among these, WAY-100635 revealed to be an important pharmacological tool in defining the 5-HT<sub>1A</sub> receptor functions. It has been demonstrated that WAY-100635 lacks of intrinsic activity in multiple assay systems that are sensitive to the effects of 5-HT<sub>1A</sub> agonists. Accordingly, WAY-100635 antagonizes the responses of 8-OH-DPAT at 5-HT<sub>1A</sub> somatodendritic autoreceptors to inhibit the firing rate in the DRN and antagonizes the ability of 8-OH-DPAT to decrease the accumulation of cAMP at postsynaptic 5-HT<sub>1A</sub> receptors in the hippocampus [36]. Although WAY-100635 has been extensively used in determining the consequences of 5-HT<sub>1A</sub> receptor blockade, it is not orally active and has a relatively short half-life that has precluded its clinical development [37]. Nonetheless, WAY-100635 has served in multiple preclinical studies to support the hypothesis that 5-HT<sub>1A</sub> receptor antagonists improve performance in cognitive tasks. For example, WAY-100635: a) attenuates the impairment of spatial learning caused by the intrahippocampal administration of scopolamine, 7-chlorokynurenic acid, or MK-801, in rats [34]; b) reverses the choice accuracy deficit in nucleus basalis magnocellularis-lesioned rats, but does not shorten correct response latencies [38]; c) reverses 8-OH-DPAT induced deficits in a rat recognition memory task [39]; d) shows pro-cognitive effects in water maze and passive avoidance in rats [40] and passive avoidance in mice [41].

The selective 5-HT<sub>1A</sub> receptor antagonists lecozotan (Fig. 6) is currently under investigation in advanced phase II clinical trials because it demonstrated in vivo its positive role in relevant models for cognition [42, 43].

Lecozotan displayed high binding affinity at the h5-HT<sub>1A</sub> receptor both using the 5-HT<sub>1A</sub> agonist [{}^{3}H]8-OH-DPAT and the 5-HT<sub>1A</sub> antagonist [{}^{3}H]WAY-100635 (K<sub)i</sub> = 1.6 and 4.5 nM, respectively). Lecozotan proved to be >100-fold selective for 5-HT<sub>1A</sub> receptors compared to over 50 different receptors, ion channels and transporters, with the exception of dopamine D<sub>4</sub> receptors, where lecozotan displayed moderate affinity (K<sub>i</sub> = 98 nM, 61-fold selectivity). Most notably, lecozotan was not active at various α-adrenergic (1A, 1B, 2A, 2B, and 2C subtypes), β-adrenergic (1 and 2), adenosine (1-3), dopamine (1, 2, 3, and 5), histamine (1-3), muscarinic (1-5), or serotonin (1B, 1D, 2A, 2C, and 3-7) receptors. In vitro 5-HT<sub>1A</sub> functional activity of lecozotan has been assessed by two methods: a) inhibition of forskolin-induced cAMP production induced by 8-OH-DPAT; b) inhibition of 8-OH-DPAT induced binding of [{}^{35}S]GTPγS to the 5-HT<sub>1A</sub> receptor/G protein complex. In both assays, lecozotan alone did not induce any agonist-like activity. The antagonistic activity of lecozotan has been confirmed in vivo. In a microdialysis assay using conscious rats, 8-OH-DPAT induced a decrease in extracellular levels of 5-HT in the hippocampus. Pretreatment with lecozotan completely attenuated the response to 8-OH-DPAT. Treatment with lecozotan alone did not produce any agonistic effects, confirming the lack of intrinsic activity even at high doses. The lack of agonistic activity in this assay, indicated that lecozotan acted as a full presynaptic antagonist. In another assay, pretreatment with lecozotan significantly antagonized the inhibition of DRN neuronal firing produced by 8-OH-DPAT. Again, lecozotan alone did not produce changes in DRN firing, indicating antagonistic activity also at postsynaptic 5-HT<sub>1A</sub> receptors. Moreover, pharmacological studies using fixed ratio responding in rats and squirrel monkeys demonstrated that lecozotan was a potent and competitive antagonist in vivo. Lecozotan also demonstrated oral activity in this model, producing a parallel rightward shift in the 8-OH-DPAT dose/response curve.

Lecozotan demonstrated cognition enhancing effect in a number of animal models of learning and memory. Treatment with lecozotan prior testing completely reversed the cognitive deficits induced by the uncompetitive NMDA antagonist MK-801 in both visual and visuospatial discrimination tasks in marmosets. Lecozotan alone had no effect. The effect of lecozotan on cognitive performance has been also examined in aged rhesus monkeys using a delayed match-to-sample task. Treatment with lecozotan resulted in a significant improvement in task accuracy above baseline (16.5%).

Currently, lecozotan is in Phase II clinical trials for Alzheimer Disease’s [44]. Very recently, the potent and selective 5-HT<sub>1A</sub> receptor silent antagonist WAY-101405 (Fig. 6) endowed with similar cognition enhancer properties as lecozotan has been reported [45].

**PARKINSON’S DISEASE AND RELATED DISORDERS**

As already mentioned above, some atypical antipsychotics shared the common feature of agonistic activity at 5-HT<sub>1A</sub> receptor. These compounds were characterized by low incidence of motor side-effects caused by activation of dopamine D<sub>2</sub> receptors. On such basis, it was hypothesized that 5-HT<sub>1A</sub> activation alone or in combination with activity at D<sub>2</sub>-like receptors could be relevant for drugs to treat abnormal involuntary movements (dyskinesia) in Parkinson’s disease (PD) or for antiparkinsonian agents with reduced side-effects, respectively.

It is well recognized that serotoninergic system dysfunction and reduced serotonin concentrations occur in the basal ganglia of patients with PD [46]. In animal models of PD, striatal dopaminergic mechanisms can be influenced by drugs interacting with serotoninergic neurons, including 5-HT<sub>1A</sub> receptor agonists [47]. In patients with advanced PD, striatal serotoninergic terminals serve as an important site for the decarboxylation of exogenous levodopa to dopamine (DA) [48]. Consequently, 5-HT<sub>1A</sub> receptor agonists act at striatal serotoninergic terminals to modify the level of DA produced by levodopa treatment. In addition, clinical and preclinical observations suggest that an increase in serotoninergic transmission can contribute to the appearance of dyskinesias [49-51]. These considerations have suggested that stimulation of striatal 5-HT<sub>1A</sub> autoreceptors might benefit extrapyramidal dysfunction and, in particular, ameliorate the motor complications associated with levodopa therapy.

In recent years, sarizotan and SLV 308 (Fig. 5), two compounds endowed with agonistic activity at 5-HT<sub>1A</sub> receptor and activity at dopamine D<sub>2</sub>-like receptors (D<sub>2,4</sub>), entered preclinical trials. In particular, sarizotan has been proposed as antidiskynetic agent, whereas SLV 308 as antiparkinsonian drug with limited motor side-effects.
Sarizotan showed affinities in the nanomolar range for the 5-HT1A receptors (rat IC50 = 6.5 nM; human IC50 = 0.1 nM), D2 (rat IC50 = 15.1 nM; human IC50 = 17 nM), hD3 (IC50 = 6.8 nM) and hD4.2 (IC50 = 2.4 nM) receptors. The affinity of sarizotan for dopamine D1 receptors as well as for the other 5-HT receptor subtypes was at least two orders of magnitude lower (except human 5-HT1 receptor: IC50 = 10 nM). Affinities to about 50 other receptors, ion channels and transporters were found to be at least >1000 nM.

The intrinsic activity of sarizotan has been assessed in comparison with 8-OH-DPAT in two different assays. Sarizotan and 8-OH-DPAT prevented in a concentration-dependent way forskolin-stimulated elevation of the intracellular cAMP level. In particular, sarizotan showed EC50 = 1.5 nM; Emax = 100% at 1 μM. It is noteworthy that sarizotan displayed efficacy (Emax) comparable to that of 8-OH-DPAT, and about six- to tenfold higher potency. In the guinea pig ileum preparation, sarizotan and 8-OH-DPAT concentration-dependently inhibited the electrically-induced contractions with IC50 values of 150 nM (Emax = 40% at 1 μM) and 580 nM (Emax = 46% at 10 μM), respectively.

Sarizotan displays partial DA agonist profile in vivo. In intact rats, sarizotan increased striatal DOPA accumulation, an indicator of DA receptor blocking properties. In reserpinized rats, sarizotan decrease DOPA accumulation in the striatum. Although the effect is modest as compared with that of dopamine full agonists, this decrease indicates that sarizotan have some level of intrinsic dopaminergic activity [52].

The ability of sarizotan to affect the response alterations complicating levodopa treatment of PD has been assessed in animal models of parkinsonism by Bibbiami et al. [53] In 6-OH-DA-lesioned rats, sarizotan had no effect on the acute rotational response to levodopa but did attenuate the shortening in motor response duration induced by chronic levodopa treatment. In MPTP-lesioned monkeys, sarizotan alone had no effect on parkinsonian severity or on the antiparkinsonian response to levodopa. In contrast, sarizotan elicited a considerable reduction of levodopa-induced choreiform dyskinesias. In both species, the motoric effects of sarizotan were blocked by WAY-100635, indicating that the observed responses were most likely mediated by stimulation of 5-HT1A autoreceptor. Moreover, the authors argued that the antidyskinetic action of sarizotan was unlikely to reflect D2-like receptor blockade because a functionally significant effect at these sites was incompatible with the absence of any reduction in the antiparkinsonian action of levodopa as well as the ability of WAY-100635 to block motoric responses to sarizotan. Furthermore, sarizotan was found unable to reduce the antiparkinsonian and dyskinesiogenic effects of the D2 agonist quinpirole alone, thus it did not exert inhibitory effects at D2 or D3 receptors.

However, Kuzhikandathil & Bartoszyk [54] pointed out that the above behavioral effects of sarizotan were not directly determined by using D2-like dopamine receptor-specific antagonists. Therefore, the authors characterized in detail the functional profile of sarizotan on D2S, D2L, D3, D4.2 and D4.4 dopamine receptors, individually expressed in AtT-20 neuroendocrine cell line. This cell line is an ideal heterologous expression system for studying D2-like dopamine receptors since it natively expresses many of the effector molecules that couple to the different D2-like receptors and also exhibits neuronal properties. In particular, the authors evaluated the effect of sarizotan on two different signaling pathways: a) the D2-like receptor-mediated inhibition of adenylyl cyclase and b) the D2-like receptor-mediated activation of G-protein coupled inward rectifier K+ channels (GIRK). It was determined that, depending on the receptor subtype and signaling pathway, sarizotan was both a partial agonist and a full agonist (Table 3). In particular, using the coupling of D2-like receptors to adenylyl cyclase, sarizotan displayed a full agonist profile at D2L, D3, D4.2, and D4.4 receptors but a partial agonist one at D2S receptors. When using the coupling of D2-like receptors to GIRK, sarizotan acted as full agonist at D3 and D4.4 receptors but as partial agonist at D2S, D2L, and D4.2 receptors. Consistent with its partial agonist property, sarizotan acted as an antagonist at D2S and D2L receptors (IC50 = 52 and 121 nM, respectively). The authors suggested that this overall profile might indicate a role for D2-like receptor, in addition to 5-HT1A receptor, in mediating the beneficial effects of sarizotan in treatment-induced dyskinesia. However, further experiments will be necessary to elucidate the relative contributions of the 5-HT1A, D2, D3 and D4 components in sarizotan’s mechanism of action.

Sarizotan underwent Phase Ia and Iib clinical trials in PD patients with dyskinesias, showing significant increases in hours of on without dyskinesia and significant reduction in hours of on with troublesome dyskinesia [55, 56], then

| Table 3. Inhibition of cAMP Levels and GIRK Current Responses Induced by Sarizotan.a |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| D2-like receptors | EC50, nM | % maximal response | EC50, nM | % maximal response |
| D2s               | 0.60 | 66 | 29 | 24 |
| D2L               | 0.51 | 100 | 23 | 42 |
| D3                | 0.47 | 83 | 5.6 | 76 |
| D4.2              | 0.48 | 98 | 4.5 | 68 |
| D4.4              | 0.23 | 89 | 5.4 | 82 |

aData taken from Ref. [54].
progressed to Phase III clinical trials. On June 2006, Merck KGaA announced that the Phase III studies did not confirm the Phase II findings nor the results from preclinical studies, therefore Merck did not plan to pursue further development of this compound [57].

In 2005, a press release announced that Solvay Pharmaceuticals advanced in Phase III clinical trials the mixed D2/D3/5-HT1A agent SLV308. This compound, which combines high potency partial agonism at dopamine D2 and D3 receptors with full efficacy low potency 5-HT1A receptor agonism, has been studied in vivo models of PD.

Dopaminergic therapies, including levodopa and dopamine agonists, are the pillars of PD therapy. However, they are associated with a high probability of motor complications (dyskinesias and motor fluctuations), psychotinic-like symptoms such as hallucinations (probably due to the over-stimulation of extra-striatal dopamine receptors), orthostatic hypotension, somnolence, and other side-effects [58, 59].

SLV308 has been developed on the basis of a multitarget strategy to obtain antiparkinsonian drugs devoted to motor side-effects. It was hypothesized that partial dopamine D2 and D3 receptor agonists should not induce motor side-effects because partial agonists would hypothetically be capable of stimulating D2 and D3 receptors when the dopaminergic tone is low, while being able to counteract excessive stimulation of the D2 and D3 receptors when the dopaminergic tone is high [60]. Moreover, the 5-HT1A receptor agonist component could ameliorate the induction of dyskinesia [61, 62].

SLV308 bound with high affinity to D2-like receptors (KD = 8.1; KD = 8.6; KD = 7.8), adrenergic receptors (rat cortex, KD = 7.8), and h5-HT1A receptors (KD = 8.5). SLV308 demonstrated moderate binding at 5-HT1A receptors (KD = 7.2), but little-no affinity at a variety of receptors, ion channels, and transporters.

In vitro experiments demonstrated that SLV308 possessed partial agonist effect at dopamine D2 and D3 receptors. Intrinsically activity of SLV308 at hD3 receptors has been assessed using the ability of the D3L receptor to attenuate forskolin-stimulated cAMP accumulation. SLV308 showed partial efficacy up to 50% at D3L receptors as compared to quinpirole (pEC50 = 8.0). SLV308 only partially antagonized the effect of quinpirole (pA2 = 8.4) in the forskolin-stimulated accumulation of cAMP. Intrinsically activity of SLV308 alone and antagonistic activity vs. DA at hD3 receptors was assessed by monitoring [35S]GTPγS binding. In this assay, the efficacy of SLV308 was approximately 67% of that of DA but with a higher potency compared to quinpirole (pEC50 = 9.2). The partial agonist nature of SLV308 at hD3 receptors was confirmed by the antagonism of the DA induction of [35S]GTPγS binding (pA2 = 9.0). The intrinsic activity of SLV308 at D4 receptors was evaluated by the D4 receptor-mediated attenuation of PGE-1 stimulated induction of alkaline phosphate secretion. SLV308 was found to exert potent (pEC50 = 8.6) but incomplete agonist activity at D4 receptors as compared to quinpirole. The intrinsic activity of SLV308 was confirmed by ex vivo neurochemistry studies (striatal forskolin-evoked cAMP production and [3H]dopamine release). Finally, SLV308 was found to activate h5-HT1A receptors as 8-OH-DPAT because it completely and in a concentration-dependent manner attenuated forskolin-induced cAMP formation in CHO cells (pEC50 = 6.3).

SLV308 showed efficacy in animal models of PD, lowering spontaneous activity when tested on locomotor behaviour in open field tests. Also, SLV308 stimulated turning behavior in rats with unilateral 6-OH-DA lesions of the substantia nigra pars compacta and submaximally (vs. quinpirole) decreased diastolic dopamine levels in the rat nucleus accumbens. In MPTP-treated marmosets SLV308 produced marked and long-lasting antiparkinsonian effects. Finally, 5-HT1A receptor agonism in vitro did translate into 5-HT1A mediated behavior in vivo (forced swim test, stress induced ultrasonic pup vocalization) [63, 64].

No clinical data on SLV308, nor any insight on the relative contribution of the D2, D3, and 5-HT1A components in mechanism of action of SLV308 are available to date.

SLV308 (pardoprunox) is currently being studied in five clinical studies for treatment of early stage PD [65].

**PAIN TREATMENT**

In a paper published in 1990, Millan & Colpaert reported that 5-HT1A partial agonists attenuated morphine-evoked antinociception, revealing an interaction between opioid and 5-HT1A receptors in the control of nociception [66]. Starting from this observation, Colpaert and coworkers [67, 68] developed the new concept that in nociceptive systems any input causes two effects that are opposite in sign. Opioid receptor activation produces both analgesia as a so-called first-order effect and hyperalgesia as a second-order effect. Upon chronic exposure to opioid, the second-order hyperalgesia grows and neutralizes the first-order effect, thus offering a description of the neuroadaptive tolerance and sensitization that develops with opioids. According to this concept, 5-HT1A receptor activation produces dual, bidirectional effects that should amount to the mirror opposite of those produced by opioids. Therefore, stimulation of peripheral nociceptors would initially produce pain as a first-order effect, but also hyperalgesia as a second-order effect; with cronicity, this second order hyperalgesia counteract the first order pain and remarkably develop into increasingly powerful analgesia.

The identification of F-13640 (Fig. 5), a highly selective and high efficacy 5-HT1A agonist enabled to prove this concept, evidencing that pro- and antinociceptive actions of 5-HT1A ligands depend on their efficacy at 5-HT1A receptor [69].

In 1999, Vacher and coworkers at Pierre Fabre SA reported on the design, synthesis, and preliminary pharmacological characterization of a series of 6-substituted-pyridin-2-ylmethylamine derivatives [70]. Particular attention was given to metabolic stability of the new series of 5-HT1A agonists, to ensure the identification of potent 5-HT1A agonists suitable for in vivo studies. Among the reported compounds, F-13640 displayed the most relevant pharmacological profile. In radioligand binding experiments, F-13640 displaced [3H]8-OH-DPAT from rat and human 5-HT1A receptors (pKi = 9.07 and 9.49, respectively).
F-13640 was found highly specific, displaying IC$_{50}$ values $>1000$ nM for various receptors (serotonergic: 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_4$, h5-HT$_6$, 5-HT$_7$; dopaminergic: D$_1$, D$_2$; adrenergic: $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$; GABA-ergic: GABA-A, GABA-B; benzodiazepine; opiate: $\mu$, $\kappa$, $\delta$; histaminergic: H$_1$, H$_3$; acetylcholinergic: muscarinic, nicotinic; adenosine: A$_1$, A$_2$; excitatory amino acid receptors: AMPA, kainate, NMDA, PCP), ion channels (Ca$^{2+}$, K$^+$, Na$^+$), uptake sites (5-HT, DA, noradrenaline), and its ability to inhibit different enzymes.

F-13640 stimulated [${}^{35}$S]GTP$_\gamma$S binding in Cos-7 cells expressing a fusion protein between recombinant h5-HT$_{1A}$ receptor and GalCys$_{351}$Gly mutant protein, activating the 5-HT$_{1A}$ receptor to an exceptionally large extent (90%). The compound was further studied in another assay system that best resolves very high levels of 5-HT$_{1A}$ receptor activation (stimulation of [${}^{35}$S]GTP$_\gamma$S binding in C6-glial cells). In this system, maximal stimulation of [${}^{35}$S]GTP$_\gamma$S binding amounted to 75% (100%: 10$^{-5}$ M 5-HT). Thus, F-13640 appeared to activate 5-HT$_{1A}$ receptors to a magnitude larger than that observed with other non-native, selective ligand [69].

The hyper- and hypalgesic effects of F-13640 were studied in normal rats and in the formalin model of tonic nociceptive pain, respectively [69]. F-13640 produced both hyperalgesia and analgesia, in a direct function of the extent of 5-HT$_{1A}$ receptors activation. With this respect, the authors emphasized the importance of the very-high-efficacy 5-HT$_{1A}$ receptor activation elicited by F-13640.

F-13640 revealed to exert an analgesic action in rat models of acute, tonic and chronic nociceptive pain that was rivaled only by large doses of high-efficacy $\mu$-opioid receptor agonists [71]. In models of neuropathic allodynia of peripheral or central origin, chronic F-13640 administration caused an analgesia that surpasses that observed with morphine, ketamine, imipramine and gabapentin. F-13640 was shown to produce long-lasting, preemptive and curative-like actions in neuropathic allodynia. The Annual Report 2005 by Pierre Fabre referred that F-13640 was being studied in Phase II clinical trials [72]. No clinical data in humans for this compound are available to date.

**CONCLUDING REMARKS**

The serotonin 5-HT$_{1A}$ receptor is likely the most studied subtype among the serotonergic receptors. Over the years, the intense research by medicinal chemists has allowed the identification of various classes of 5-HT$_{1A}$ receptor ligands. The use of some prototypical 5-HT$_{1A}$ agonists and antagonists has led to meaningful insight into the pathophysiological role of 5-HT$_{1A}$ receptor. It became soon apparent that 5-HT$_{1A}$ receptor was involved in anxiety and depression and also that activation of this receptor would have beneficial effect on some unwanted side-effects of antipsychotic drugs. In recent years, the identification of some potent and selective 5-HT$_{1A}$ receptor agents led to the definition of newer therapeutic areas wherein the 5-HT$_{1A}$ receptors were involved. Among the selective agonists, repinotan and piclozotan demonstrated anti-ischemic properties, whereas F-13640 showed remarkable analgesic properties. The 5-HT$_{1A}$ agonist component of sarizotan and SLV308 has been indicated to have beneficial effect on levodopa-induced dyskinesia. On the other hand, the antagonist lecozotan has demonstrated cognition enhancing effects. A key aspect emerging from the studies on repinotan and F-13640 is the in-depth characterization of agonist intrinsic activity. In fact, many 5-HT$_{1A}$ agonists have been characterized only in comparison with 8-OH-DPAT which show, at least in some biological systems, partial agonist properties as compared to the native agonist 5-HT. Therefore, the evaluation in different in vitro and in vivo models of 5-HT$_{1A}$ receptor activation will give an accurate characterization of agonistic properties.

In recent years, early ADMET studies have been employed in order to significantly reduce the failure rate in the development of drug candidates. This concept has been also applied in serotonin 5-HT$_{1A}$ receptor field, leading to lecozotan, the first orally active silent antagonist. Similarly, Epix Pharmaceuticals rapidly progresses in Phase 2b clinical trials for major depressive disorders the potent and selective agonist PRX-00023 [73].

Although some of the discussed agents will not reach the market as new drugs, they are already revealing as powerful tools for elucidation of molecular mechanisms in which the 5-HT$_{1A}$ receptor is involved. Therefore, it will not be surprising if other newer therapeutic indications will be hypothesized in the near future based on the studies performed on agents that today have failed to reach the market. This has already begun as witnessed by a number of patents claiming 5-HT$_{1A}$ agonists useful for treatment of sexual disorders. Flibanserin, a 5-HT$_{1A}$/5-HT$_{2A}$ agent, previously proposed as antidepressant, is currently in Phase III clinical trials for treatment of hypo sexual desire disorders [74].

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DRN</td>
<td>Dorsal raphe nucleus</td>
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<tr>
<td>GAD</td>
<td>Generalized anxiety disorder</td>
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<tr>
<td>GIRQ</td>
<td>G-protein coupled inward rectifier K$^+$ channel</td>
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<tr>
<td>5-HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>p-MCA-O</td>
<td>Permanent middle cerebral artery occlusion</td>
</tr>
<tr>
<td>t-MCA-O</td>
<td>Transient middle cerebral artery occlusion</td>
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<tr>
<td>MPTP</td>
<td>1-Methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine</td>
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<tr>
<td>8-OH-DPAT</td>
<td>8-Hydroxy-2-(di-N-propylamino) tetralin</td>
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<tr>
<td>PD</td>
<td>Parkinson disease</td>
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<tr>
<td>PTBBS</td>
<td>Peripheral type benzodiazepine binding site</td>
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<tr>
<td>aSDH</td>
<td>Acute subdural ematoma</td>
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<tr>
<td>TBI</td>
<td>Traumatic brain injury</td>
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5-HT1A Receptor, an Old Target for New Therapeutic Agents

REFERENCES


