

Serotonergic 5-HT_{2C} Receptors as a Potential Therapeutic Target for the Design Antiepileptic Drugs

Methvin Isaac*

NPS Pharmaceuticals Inc., 6850 Goreway Drive, Mississauga, ON, Canada, L4V 1V7

Abstract: A variety of clinical observations suggest that certain forms of epilepsy are due to long-term, progressive changes in neural networks that eventually provoke spontaneous and recurring seizures. Recently, there has been growing evidence that serotonergic neurotransmission modulates experimentally induced seizures and is involved in the enhanced seizure susceptibility observed in some genetically epilepsy-prone animals. Generally, agents that elevate extracellular serotonin (5-Hydroxytryptamine, 5-HT) levels, such as 5-hydroxytryptophan, and 5-HT reuptake blockers inhibit both limbic and generalized seizures. Conversely, depletion of brain 5-HT lowers the threshold to audiogenically, chemically and electrically-evoked convulsions. More specifically, the recent finding that the 5-HT_{2B/2C} receptor agonist, 1-(m-chlorophenyl)-piperazine (mCPP) is anticonvulsant has kindled an interest into the investigation of the serotonergic 5-HT_{2C} receptor subtype as a potential target for the treatment of epilepsy. Further pharmacological evaluation of selective activation or inactivation of the 5-HT_{2C} receptor subtype with selective agonist/positive modulators and antagonists will provide important information about the therapeutic contribution of this receptor to the epileptic circuitry in the brain. Future development of serotonergic antiepileptic drugs will be a significant addition to the therapeutic armamentarium against epilepsy.

Key Words: Serotonergic receptors, Epilepsy, antiepileptic drugs, selective 5-HT_{2C} agonist.

INTRODUCTION

Epilepsy, a common neurological disorder characterized by recurrent spontaneous seizures, is considered to be a major health problem that affects approximately one to two percent of the population worldwide [1]. Epilepsy also poses a considerable economic burden on society. The direct costs of epilepsy vary significantly depending on the severity of the disease and the response to treatment. Despite the considerable progress in our understanding of the pathophysiology and pharmacotherapy of seizures and epilepsy [2], the cellular basis of human epilepsy remains an enigma. In the absence of etiological understanding, approaches to pharmacotherapy must be directed to the control of symptoms, that is the suppression of seizures. More concerning is that current antiepileptic drugs do not halt the underlying natural progression of the disorder.

Over the years, there has been considerable success in the development of novel antiepileptic drugs (AED) along with new improved formulations. These include older 'first generation' drugs such as carbamazepine, phenobarbital, valproic acid and newer, 'second generation' drugs such as lamotrigine, vigabatrin, tiagabine, topiramate, gabapentin and levetiracetam [3, 4]. The selection of an antiepileptic drug for treatment is predicated on its efficacy for the specific type of seizures, tolerability and safety [5, 6].

Epileptic seizures can be either generalized (generalized epileptic seizure), originating in both hemispheres of the brain simultaneously, or partial (focal seizures) originating in

one or more parts of one or both hemispheres, most commonly the temporal lobe. With generalized seizures, consciousness is always impaired or lost. Consciousness may be maintained in partial seizures but partial seizures may become generalized seizures in a process referred to as secondary generalization, at which point consciousness is lost. In patients the type of epilepsy or epileptic syndrome are further classified according to features such as the type of seizure, etiology, age of onset and electroencephalogram. Epilepsy or epileptic syndromes can be either idiopathic (etiology or cause is unknown) with a presumed genetic basis or symptomatic (acquired). The known potential causes of epilepsy include brain tumors, infections, traumatic head injuries, perinatal insults, developmental malformations, cerebrovascular diseases, febrile seizures and status epilepticus [7].

Traditionally, pharmacological strategies for treatment of epilepsy are aimed at suppressing either the initiation or propagation of seizures rather than the underlying processes that lead to epilepsy. Some epileptic patients are unresponsive to current antiepileptic drug treatment and for this reason the major goal in epilepsy research has been to develop drugs with greater anticonvulsant efficacy and less toxicity than existing drugs [8]. There is growing evidence that serotonergic neurotransmission modulates a wide variety of experimentally-induced seizures and is involved in the enhanced seizure susceptibility observed in some genetically epilepsy-prone animals [9-14]. This review highlights the developments in the knowledge of the mammalian 5-HT_{2C} receptor subtypes, specifically its structure, pharmacology, CNS distribution and actions at the molecular level. However, particular emphasis or focus will be on the therapeutic potential of this molecular target in the design of antiepileptic drugs.

*Address correspondence to this author at NPS Pharmaceuticals Inc., 6850 Goreway Drive, Mississauga, ON, Canada, L4V 1V7; Tel.: +905-677-0831; Fax: 905-677-9595; E-mail: misaac@nps.com

5-HT_{2C} RECEPTOR STRUCTURE, DISTRIBUTION AND FUNCTION

5-Hydroxytryptamine (5-HT or Serotonin), a key neurotransmitter of the peripheral and central nervous system (PNS and CNS), has been implicated in a variety of sensory, motor and behavioral processes. The diverse effects of this neurotransmitter are related to the extensive projections of serotonergic neurons throughout the brain and the large number of distinct serotonin receptor subtypes. At least 14 distinct serotonin receptor subtypes are expressed in the mammalian CNS, each of which is assigned to one of seven families, 5-HT₁ to 5-HT₇ (Figure 1).

The 5-HT₂ sub-family of serotonin receptors is composed of three subtypes, the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. All three receptors are G-protein coupled to the activation of the phospholipase C (functionally linked to phosphatidyl inositol (PI) hydrolysis) and subsequent mobilization of intracellular calcium [15].

The 5-HT_{2C} receptor was identified as a tritiated-5-HT binding site in the choroid plexus (tissue involved in production of cerebrospinal fluid, CSF) of various species that could also be labeled by tritiated -mesulergine and tritiated-lysergic acid diethylamide (LSD). Originally this site was seen as a new member of the 5-HT₁ receptor family, and termed 5-HT_{1C}, because of its high affinity for tritiated 5-HT [16]. However, once the receptor was cloned and more information about its characteristics became available, a shift to the 5-HT₂ receptor family and reclassification as 5-HT_{2C} receptors became accepted [17].

5-HT_{2C} Receptor Structure

The partial cloning of the mouse 5-HT_{2C} receptor [18] was shortly followed by the sequencing of the full length

clone in the rat [19], mouse [20] and human [21]. Additionally, a splice variant of the 5-HT_{2C} receptor has been observed in brain tissues of the rat, mouse and human [22]. The functional significance of this variant is however, unclear as the protein product is truncated and lacks a 5-HT binding site. More recently, it has been reported that 5-HT_{2C} mRNA undergoes post-transcriptional editing to yield multiple 5-HT_{2C} receptor isoforms with different distributions in brain. In functional terms, this is potentially of great significance as the amino acid sequences predicted from the mRNA transcripts indicate that the isoforms (if expressed endogenously in significant amounts in brain tissue) may have different regulatory and pharmacological properties [23].

The gene for the 5-HT_{2C} receptor is located on the human X chromosome at position q 24 (Xq24). The 5-HT_{2C} receptor gene has three introns (rather than two as in the case of the 5-HT_{2A} and 5-HT_{2B}) and may produce a protein product with eight rather than seven transmembrane regions, which, if proven, would be unusual for a G-protein coupled receptor [20]. There is high sequence homology (>80% in the transmembrane regions) between the mouse, rat and human 5-HT_{2C} receptors. The mouse and rat 5-HT_{2C} receptors possess six potential N-glycosylation sites, four of which are conserved in the human sequence. The rat 5-HT_{2C} receptor has eight serine/threonine residues representing possible phosphorylation sites, all of which are conserved in the human sequence [15].

5-HT_{2C} Receptor Distribution

In contrast to the 5-HT_{2A} receptor (expressed in CNS and PNS tissues) and 5-HT_{2B} receptor (expressed principally in the periphery and only sparsely in the CNS), the 5-HT_{2C} receptor has been found primarily in CNS.

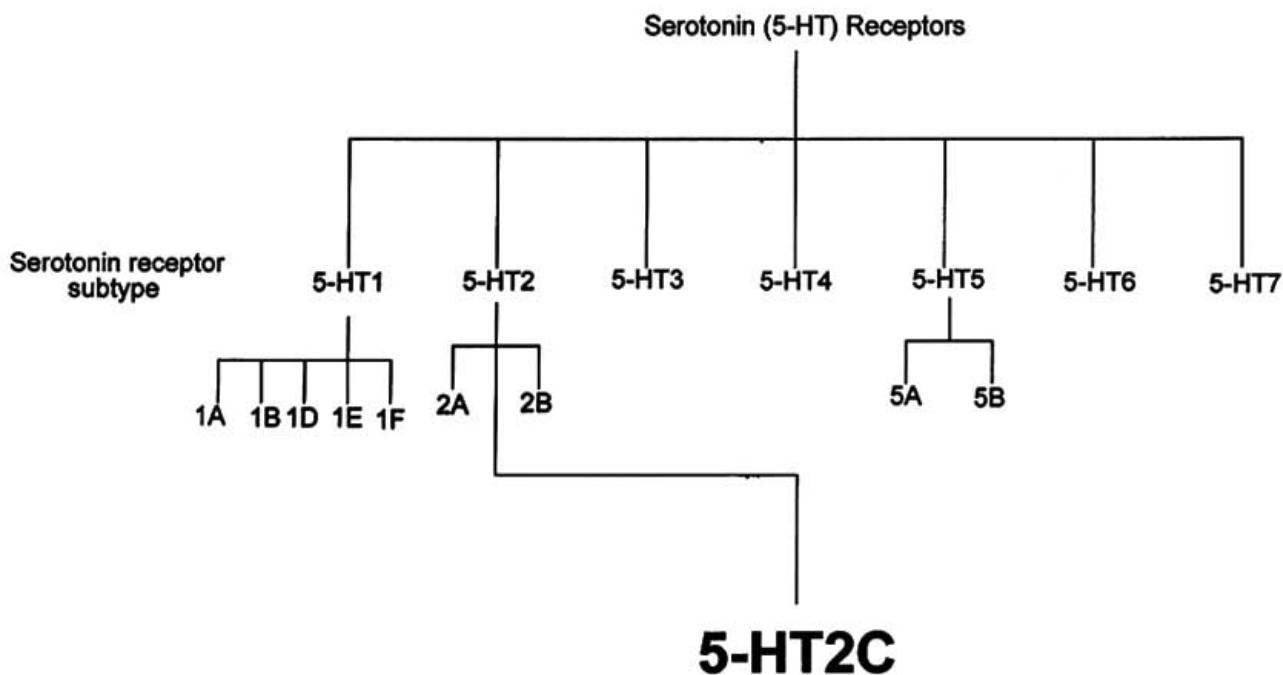


Fig. (1). A schematic showing the 14 serotonin receptor subtypes highlighting simplistically the subtype relationships.

Autoradiographic studies, using a variety of ligands (including tritiated-5-HT, tritiated-mesulergine and tritiated-LSD), have provided a detailed map of the distribution of 5-HT_{2C} binding sites in rat and many other species [24, 25]. In addition to the very high levels detected in the choroid plexus, 5-HT_{2C} binding sites are widely distributed and present in the cortex (olfactory nucleus, pyriform, cingulate and retrosplenial), limbic system (nucleus accumbens, hippocampus, amygdala) and the basal ganglia (caudate nucleus, substantia nigra). The presence of 5-HT_{2C} binding sites in the pyriform cortex and substantia nigra is relevant to findings of 5-HT_{2C} receptor-mediated electrophysiological responses in these regions [26, 27].

Two studies have reported 5-HT_{2C} receptor mRNA in the midbrain raphe nuclei [28, 29]. In addition, both 5-HT_{2C} receptor mRNA and immunoreactivity have been found in the central grey which is adjacent to the dorsal raphe nucleus (DRN). Experimental findings to date seem to suggest that there is a good concordance between the distribution of 5-HT_{2C} receptor mRNA and 5-HT_{2C} binding sites. Furthermore, neurotoxic lesion experiments indicate that the 5-HT_{2C} receptors are mostly post-synaptic but there is also evidence suggesting possible presynaptic localization on 5-HT nerve terminals. Thus, whilst the 5-HT_{2C} receptors are clearly located post-synaptically, the possibility of a presynaptic location needs further study [30].

Functional Effects Mediated Via the 5-HT_{2C} Receptor

Signal Transduction

Agonist binding to the 5-HT_{2C} receptor, activates phospholipase C via activation of a G protein (Gq11). Phospholipase C catalyzes the hydrolysis of phosphatidylinositol-4, 5-bisphosphate to inositol 1,4,5-triphosphate and diacylglycerol. Inositol 1,4,5-triphosphate, acting as a second messenger, diffuses through the cell cytoplasm and stimulates the release of calcium sequestered in the endoplasmic reticulum which in turn activates numerous cellular processes through the intermediacy of calmodulin and its homologs. The diacylglycerol remains associated with the plasma membrane where it activates protein kinase C to phosphorylate and thereby modulate the activities of a number of cellular proteins. In choroid plexus preparations, the non-selective 5-HT₂ receptor agonists, mCPP, TFMPP, and MK-212 behave as agonists but only the latter compound had an efficacy equal to 5-HT. It has been suggested that 5-HT_{2C} receptors in choroid plexus may regulate CSF formation as a result of their ability mediate cyclic guanosine monophosphate (cGMP) formation [31-34].

To further determine the intracellular mechanism of the 5-HT_{2C} receptor endogenously expressed in the choroid plexus epithelial cells, an elegant strategy of targeted disruption of protein-protein interactions was recently employed [35]. The strategy entails the delivery of conjugated membrane-permeable peptides that disrupts domain interaction at specific steps in the signaling cascade. For example, peptides targeted against receptor Galpha q-protein interaction domain were found to disrupt the 5-HT_{2C} receptor-mediated phosphatidylinositide hydrolysis. In contrast, peptides that bind to and sequester free Gbetagamma subunits were ineffective at blocking 5-HT_{2C}

receptor mediated phosphatidylinositol turnover. These results provide the first direct demonstration that active Galpha q subunits mediate 5-HT_{2C} receptor activation of phospholipase C beta and that the Gbetagamma subunits released from Galpha q heterotrimeric proteins are not involved.

Genetic and molecular events regulate the creation of variants of 5-HT_{2A} and 5-HT_{2C} receptors whose diversity has important functional significance. Overall sequence identity between the 5-HT_{2A} and 5-HT_{2C} receptors is quite high and it is not surprising that the mechanisms of regulation for these two receptors are similar. There is, however, one striking difference between these two receptors, which involves RNA editing (a mechanism for generating molecular diversity by altering the genetic code at the level of RNA). The 5-HT_{2C} receptor is the only known G protein-coupled receptor whose mRNA undergoes post-transcriptional editing to yield different receptor isoforms [36]. The different 5-HT_{2C} receptor isoforms generated from RNA editing have demonstrated altered dynamics of agonist-induced calcium release. These distinctions in agonist-induced calcium release imply that edited 5-HT_{2C} receptors may produce distinct physiological responses within the CNS [37]. Particular isoforms vary with regard to their central distribution and the extent to which they display constitutive activation. It has been shown that editing sites are located on the second intracellular loop, which contains a consensus sequence for G-protein interaction [38]. It is therefore clear that changes in amino acid sequence may affect the coupling ability between the receptor and its G-protein. In this regard, it was recently reported that depletion of serotonin increases expression of 5-HT_{2C} mRNA isoforms encoding receptors with higher sensitivity to serotonin. These results indicate that mRNA editing may serve as a mechanism whereby 5-HT_{2C} receptor activity is stabilized in the face of changing synaptic serotonergic input [39].

5-HT_{2C} RECEPTORS AND EPILEPSY

Epilepsy, a brain disorder manifested by recurrent seizures, refers to a complicated constellation of more than 40 distinct disorders. The seizure, a sudden massive neuronal discharge, can be either partial or complete, depending on the area of brain involved or whether or not consciousness is impaired. Normally there is a balance between excitation and inhibition in the brain. When this balance is disrupted by increased excitation or decreased inhibition, a seizure may result. The neuronal discharges may stimulate muscles innervated by the nerves involved, resulting in involuntary muscle contractions, or convulsions [40].

There are currently several drugs in clinical use to inhibit seizures, which fall into three different categories in terms of their target [41]. Most common are the drugs that affect the flow of sodium into the cell via voltage-gated sodium ion channels. A sodium ion channel is a structure in the cell membrane that is selectively permeable to sodium ions and is opened by changes in voltage across the cell membrane. Other drugs affect calcium ion channels. The third category of drugs affects some aspect of inhibitory synapses that are activated by the neurotransmitter -aminobutyric acid

(GABA). Despite the availability of these drugs, a large proportion of patients continue to have seizures. Furthermore, among those in whom seizures are effectively inhibited, substantial numbers experienced persistent and undesirable effects from these drugs. In light of this, the current goal of researchers is to identify new classes of anti-seizure drugs that act on novel molecular targets and by novel mechanisms that may permit effective treatment of large numbers of individuals unsatisfactorily treated at present. The recently cloned 5-HT_{2C} receptor has revealed a novel molecular target that provides just this opportunity for the development of novel antiepileptic drugs.

There is growing evidence that serotonergic neurotransmission modulates a wide variety of experimentally-induced seizures and involved in the enhanced seizure susceptibility observed in some genetically prone rodents [42, 43]. Studies have shown that mice bearing a targeted disruption of the 5-HT_{2C} receptor genes exhibit an epilepsy syndrome associated with sporadic spontaneous seizures that occasionally result in death. In all epileptic paradigms, mice lacking the 5-HT_{2C} receptors were significantly more seizure susceptible than wild-type controls. Results indicate that mutants have lower focal seizure thresholds, increased focal seizure excitability, and facilitated propagation within the forebrain seizure system. Mutants also exhibit lower generalized seizure threshold for the expression of both generalized clonic and generalized tonic seizures. Importantly, the 5-HT receptor antagonist, mesulergine (2 or 4 mg/kg), administered prior to electroshock testing, recapitulated the mutant phenotype in wild-type mice. Together, these data strongly implicate a role for serotonin and the 5-HT_{2C} receptors in the modulation of neuronal network excitability and seizure propagation throughout the CNS [44-46]. Furthermore, agents that elevate extracellular serotonin (5-HT) levels, such as 5-hydroxytryptophan and 5-HT reuptake blockers, inhibit both limbic and generalized seizures. Conversely, depletion of brain 5-HT lowers the threshold to audiogenically, chemically and electrically evoked convulsion [47-49].

Fluoxetine **1** (Figure 2), a selective serotonin reuptake inhibitor, has been shown to exert a protective action against convulsive seizures in animal models when administered systemically or focally into the substantia nigra [50]. A similar reduction in seizure activity has been observed for the 5-HT_{2C} receptor agonists mCPP and TFMPP when microinjected bilaterally into the rat substantia nigra. This indicates that the 5-HT_{2C} receptors in the substantia nigra may contribute to seizure regulation [51, 52]. Among the clinically effective anticonvulsants such as carbamazepine, dose-related anticonvulsant effects correlate with increased extracellular serotonin further implicating the role of serotonin and hence the 5-HT_{2C} receptor agonist in epileptic seizures. Nevertheless, cross talk between the 5-HT_{2C} and -amino butyric acid (GABA) receptors in the mediation of the observed anticonvulsant activity should not be overlooked [53].

Furthermore, Citalopram **2** (Figure 2), a more selective serotonin reuptake inhibitor when administered to non-depressed patients with poorly controlled epilepsy as an add-on therapy decreased the seizure frequency in these patients

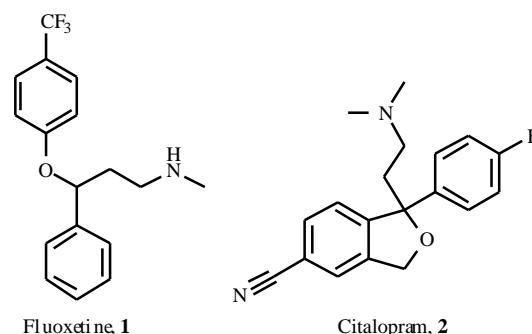


Fig. (2). Drugs that increase extracellular serotonin levels resulting in 5-HT_{2C} activation.

[54]. This result is further evidence of a serotonergic impairment, possibly 5-HT_{2C} receptors, as a possible mechanism of epilepsy. Although controlled studies are required to validate this hypothesis, the antiepileptic action of both citalopram and fluoxetine demonstrate that an anticonvulsant effect can be obtained not only by affecting the GABA and glutamate system but also by potentiating serotonergic activity.

Despite the fact that a large number of 5-HT receptors with different anatomical localization and function have been identified, there are only few studies investigating the role of 5-HT receptor subtypes in the modulation of seizure activity and the results are sometimes controversial depending on the experimental epilepsy model used [55]. In order to further delineate the role of the 5-HT_{2C} receptors in seizure generation, the effects of the 5-HT_{2C} preferring agonist, mCPP, were evaluated in a genetic absence epilepsy model. mCPP weakly elevated seizure threshold in mice (but not in rats) electroshock test, however appreciable protection against pentylenetetrazol-induced myoclonic and/or tonic seizures in mice and rats were observed. This protection against pentylenetetrazol-induced myoclonic and/or tonic seizures in mice and rats was inhibited by the 5-HT_{2C/2B} receptor antagonist, SB 206533. The fact that the 5-HT_{2B} agonist, BW-723C86, had no effect on animal seizure models supports the view that the 5-HT_{2C} receptor mediated the mCPP-induced anticonvulsant effects [56]. The selective 5-HT_{2C} receptor antagonist, SB 242084 do not induce pro-convulsant effects in rats, which are characteristic of mutant mice lacking the 5-HT_{2C} receptor. This failure to exhibit pro-convulsant properties in rats in contrast to the reported characteristics of mutant mice lacking 5-HT_{2C} receptors might be accounted for by species differences [57].

To further realize the growing potential of 5-HT_{2C} agonists as useful antiepileptic drugs, a larger number of the more selective 5-HT_{2C} agonist ligands (see Figure. 3, 4, 5) recently identified need to be evaluated in preclinical and clinical epileptic models.

5-HT_{2C} LIGANDS AS POSSIBLE TOOLS AND CANDIDATES FOR PRECLINICAL AND CLINICAL EVALUATION

The pharmacology of the 5-HT_{2C} receptor is of great interest and many of the molecular tools available to probe

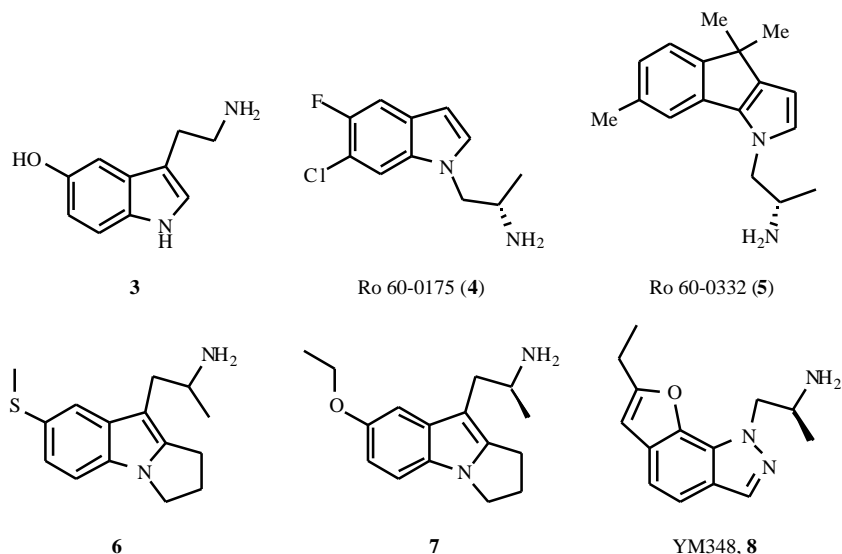


Fig. (3). Tryptamines and Isotryptamines as selective 5-HT_{2C} agonist with potential antiepileptic properties.

the consequences of 5-HT_{2C} activation in epilepsy lacked selectivity versus the other 5-HT receptors, especially the other 5-HT₂ receptor subtypes. Activation of the 5-HT_{2A} and 5-HT_{2B} has been suggested to produce undesirable physiological effects (hallucinations, platelet aggregation, vasoconstriction, pulmonary hypertension and valvulopathy). Given the physiological effects associated with 5-HT_{2A} and 5-HT_{2B} receptor activation, and the need for anti-epileptic medications with very few undesirable or intolerable effects, the search for selective 5-HT_{2C} agonists has been a major focus of research in this area. Despite the progress that has been made in the development of 5-HT_{2C} ligands, relatively few molecules have promising functional selectivity over the 5-HT_{2A} and 5-HT_{2B} receptors [58-60].

Most of the recently published 5-HT_{2C} agonist ligands bear common structural motifs with the endogenous ligand, serotonin (**3**), possessing a basic amine two or three atoms away from an aromatic core. Researchers have taken advantage of the observation that the hydroxyl group in serotonin is not critical for 5-HT_{2C} activities. For example, the isotryptamines **4** and **5** (Figure 3) were found to be potent 5-HT_{2C} ligands, however, these compounds lacked selectivity versus other 5-HT receptors, especially the other 5-HT₂ receptor subtypes, and therefore the resulting pharmacological outcomes utilizing these ligands should be assigned to 5-HT_{2C} receptor activation with appropriate caution.

Researchers at Vernalis have reported on a series of tricyclic indoles (exemplified by **6** and **7**) as 5-HT_{2C} agonists, containing a 2-aminopropyl group as a common motif, which is thought to provide improved metabolic stability (reduction of oxidative deamination) and improved selectivity over 5-HT₁ receptors [58]. Some excellent functional selectivity for 5-HT_{2C} over 5-HT_{2A} has been demonstrated for compound **6** (EC_{50} (h5-HT_{2C}) = 8 nM, 80% of the 5-HT response in a calcium mobilization assay). Unlike compound **6** that is racemic, compound **7** is chiral with the S-enantiomer being preferred. This compound

displayed good functional selectivity over 5-HT_{2A} with good potency at the 5-HT_{2C} receptors (EC_{50} (h5-HT_{2C}) = 4 nM, 87% of the 5-HT response in a calcium mobilization assay). Another functionally selective and orally active 5-HT_{2C} agonists with the potential to demonstrate antiepileptic effects is the indazole YM-348, **8** (EC_{50} (h5-HT_{2C}) = 1 nM, 76% of the 5-HT response), which showed functional selectivity in a phosphoinositide hydrolysis assay over 5-HT_{2A} (EC_{50} (h5-HT_{2A}) = 93 nM, 97% of the 5-HT response). However, very little selectivity was observed over the 5-HT_{2B} (EC_{50} (h5-HT_{2B}) = 3.2 nM, 110% of the 5-HT response) [61].

Several series of compounds are also related to mCPP **9**, TFMPP **10**, MK-212 **11** and Org-12962 **12**. These arylpiperazines are known to be 5-HT_{2C} agonists but they are not selective for the 5-HT_{2C} receptors. The anticonvulsant effect demonstrated by mCPP has given impetus to research efforts to seek out similar pharmacophores with favorable pharmacokinetic properties. Many variations on arylpiperazines have been explored in an effort to find potent agonists having promising functional selectivity over the 5-HT_{2A} and 5-HT_{2B} receptors. Biovitrum (formerly part of Pharmacia) has disclosed piperazinylopyazine **13** with excellent affinity (K_i (h5-HT_{2C}) = 8 nM) for the 5-HT_{2C} receptors although no functional data was provided [58].

Eli Lilly has reported *in vitro* and *in vivo* profiles of a selective 5-HT_{2C} receptor agonist, LY-448100 **14**. LY-448100 has an EC_{50} of 8 nM with an E_{max} 110% for the 5-HT_{2C} receptor with very good selectivity against the highly homologous 5-HT_{2A} and 5-HT_{2B} receptors. This compound also exhibited very good oral activity in rats (oral bioavailability = 83%) making it an ideal candidate for further proof of concept as an orally active anticonvulsant [60].

More recently two additional piperazine analogs **15** and **16** were disclosed as potent and selective 5-HT_{2C} agonists. These analogues are claimed to be useful as potential antiepileptics but no supporting *in vivo* data was reported [58].

Researchers at Wyeth recently disclosed a novel class of arylhomopiperazines. The specified compound **21** showed binding affinity at the h5-HT_{2C} receptor of 112 nM but no functional data was provided [58].

Finally, Hoffmann- la Roche and Vernalis reported a piperazine **22** where the basic nitrogen atom appears to be spatially distant from the aromatic core. Compound **22** possessed good potency (EC_{50} (h5-HT_{2C}) = 22 nM, 91% of the 5-HT response) and good functional selectivity over the 5-HT_{2A} receptors [58].

Further evaluation of selective 5-HT_{2C} agonists in animal models of epilepsy has to be performed to realize the full potential of selective 5-HT_{2C} agonists in anticonvulsant therapy.

Besides direct agonistic activity, 5-HT_{2C} receptor-mediated actions of serotonin may be potentiated by ligands other than receptor agonists via allosteric modulation. An allosteric modulator is a compound that affects the binding of an agonist to the primary binding site of the receptor protein by interacting with a secondary binding site, which is distant from but functionally coupled to the primary recognition site. Allosteric enhancers or positive allosteric modulators of the 5-HT_{2C} receptors present a novel drug design strategy to augment the response to endogenous serotonin in a site and event specific manner. Researchers at Pharmacia (now Pfizer) reported the discovery and characterization of PNU-69176E **23** (Figure 6), a positive allosteric modulator with high selectivity for the 5-HT_{2C} receptors [62]. PNU-69176E (L-threo- α -D-galacto-octopyranoside, methyl-7-chloro-6, 7, 8-trideoxy-6-[[[4-undecyl-2-piperidyl]carbonyl]amino]-1-thio-monohydrochloride) at 10 μ M markedly stimulated [³H]5-HT (2 nM) binding (~300%). PNU-69176E concentration-dependently enhanced the affinity and efficacy of serotonin for the 5-HT_{2C} receptors. This novel approach of using positive allosteric modulators of the 5-HT_{2C} receptors to develop novel anticonvulsant therapy is a very attractive strategy since it is much easier to achieve high receptor subtype selectivity or even absolute specificity with a ligand binding to the allosteric site than with orthosteric ligands that bind to the agonist binding site.

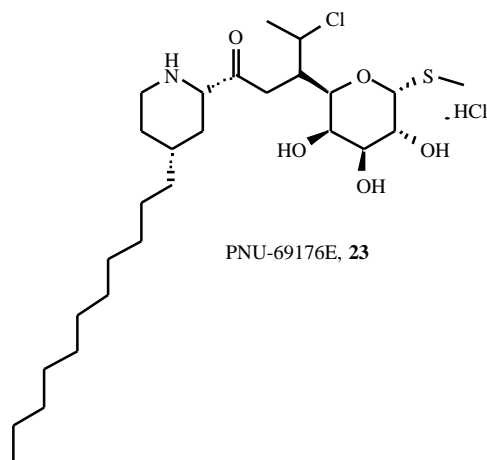


Fig. (6). PNU-69176E, a positive allosteric modulator of the human 5-HT_{2C} receptor.

CONCLUSION

Researchers have found more than 40 genes that cause epilepsy in mice or humans. There is great diversity within these 40 genes and it is thought that a large portion of the human epilepsies comprises disorders in which the inheritance of two or three susceptibility genes in the same individual is required to produce epilepsy [62].

The identification of these genes, which cause the rarer forms of epilepsy, can provide powerful clues to novel antiseizure drug mechanisms and, thus, new forms of effective antiseizure drugs. In other words, the protein coded by the mutant gene can suggest new molecules to be targeted by the antiseizure drugs. These drugs might regulate the structure and function of the molecule to have antiseizure effects. Conversely, understanding the mechanism by which these drugs act may in turn provide a clue to decoding the epilepsy genes.

Even in individuals known to be at high risk for developing epilepsy, there is currently no effective method of preventing the development of the disease. In addition, once individuals become afflicted with epilepsy, doctors have no way of curing the disease. Rather, current therapies are entirely symptomatic, analogous to the treatment of diabetes with insulin. Like the diabetic, the epileptic can take drugs that inhibit the symptoms of the disease; in this case seizures, but these drugs cannot abolish the problem entirely.

Despite the availability of new antiepileptic drugs (AEDs) with novel pharmacological modes of action, the efficacy of the medical therapies in terms of seizure control has not increased significantly over recent years. However, the new drugs have brought considerable improvement in tolerability of anticonvulsant therapy. Nevertheless, there is a continuing need for new AEDs, especially for focal and secondary generalized seizures.

The 5-HT_{2C} receptor subtype appears to be a rational target for the development of novel antiepileptic drugs. The effects seen with 5-HT_{2C} agonists are consistent with data on mutated 5-HT_{2C} receptor-deficient animals suggesting that the 5-HT_{2C} receptors may mediate tonic inhibition of neuronal network excitability. Polymorphism of the 5-HT_{2C} receptor genes has been proposed to be associated with a number of CNS disorders. In addition, the possible role of somatic mutations of 5-HT_{2C} receptors in the genetic predisposition to or pathophysiology of epilepsy cannot be excluded. Recent advances in the understanding of the biology and function of the 5-HT_{2C} receptor, along with the design and development of novel, potent and selective agonist ligands, raises the exciting possibility of an entirely novel class of antiepileptic drugs.

The available evidence suggests that the combination of AEDs with different modes of action may be more effective than monotherapy. It is therefore conceivable that AEDs with new modes of action, such as 5-HT_{2C} receptor agonism, can improve the effectiveness of medical therapy with respect to seizure control if they are sensibly combined with existing AEDs. Obviously, such 'rational polytherapy' is only possible if drug interactions do not prevent the use of the chosen agents in combination. Future clinical approval of

5-HT_{2C} agonists as AEDs is expected to be a useful addition to the treatment armamentarium for focal and secondary generalized seizures

REFERENCES

- [1] Brown, T. R.; Holmes G. L. Epilepsy. *New. Engl. J. Med.*, **2001**, *344*, 1145-1151.
- [2] McNamara, J.O. Emerging Insights into the genesis of epilepsy. *Nature*, **1999**, *399*, A15-A22.
- [3] Brazil, C. W.; Pedly, T.A. Advances in the Medical Treatment of Epilepsy. *Ann. Rev. Med.*, **1998**, *49*, 135-162.
- [4] McCabe, P. H. New Anti-Epileptic drugs for the 21st Century. *Expert Opinion. Pharmacother.*, **2000**, *1*, 633-674
- [5] Regesta, G.; Tanganelli, P. Clinical Aspects and Biological Bases of Drug-resistant Epilepsies. *Epilepsy Res.*, **1999**, *34*, 109-122.
- [6] Kwan, P.; Brodie, M. J. Early Identification of Refractory Epilepsies. *New. Engl. J. Med.*, **2000**, *342*, 314-319.
- [7] Loscher, W. C Current Status and Future Directions in the Pharmacotherapy of Epilepsy. *Trends Pharmacol. Sci.*, **2002**, *23*, 113-118.
- [8] Bauer, J.; Reuber, M. Medical Treatment of Epilepsy. *Expert Opinion. Emerging Drugs*, **2003**, *8*, 457-467.
- [9] Buterbaugh, C. G. Effect of drugs modifying central serotonergic function on the response of extensor and nonextensor rats to maximal electroshock. *Life Sci.*, **1978**, *23*, 2393-2404.
- [10] Daily, J. W.; Yan, Q. S.; Mishra, P. K.; Burger, R. L.; Jobe, P. C. Effects of fluoxetine on Convulsions and on brain serotonin as detected by microdialysis in genetically epilepsy-prone rats. *J. Pharmacol. Exp. Ther.*, **1992**, *260*, 533-540.
- [11] Filakovszky, J.; Gerber, K.; Bagdy, G. A Serotonin-1A receptor agonist and an N-methyl-D-aspartate receptor antagonist oppose each others effects in a genetic rat epilepsy model. *Neurosci. Lett.*, **1999**, *261*, 89-92.
- [12] Gerber, K.; Filakovszky, J.; Bagdy, G.; Halasz, P. The 5-HT_{1A} agonist 8-OH-DPAT increases the number of spike wave discharges in a genetic rat model of absence epilepsy. *Brain Res.*, **1998**, *807*, 243-245.
- [13] Hiramatsu, M.; Kazutoshi, O.; Kabuto, H.; Mori, A. Reduced uptake and release of 5-hydroxytryptamine and taurine in the cerebral cortex of epileptic EI mice. *Epilepsy Res.*, **1987**, *1*, 40-44.
- [14] Przegalinski, E. Monoamines and pathophysiology of seizure disorders. *Handbook of Experimental Pharmacology*, **1985**, 101-137.
- [15] Barnes, N. M.; Sharp, T. A review of central 5-HT receptors and their function. *Neuropharmacology*, **1999**, *38*, 1083-1152.
- [16] Pazos, A.; Hoyer, D.; Palacios, J.M. The binding of serotonergic ligands to the porcine choroid plexus, characterization of a new type of serotonin recognition site. *Eur. J. Pharmacol.*, **1984**, *106*, 539-546.
- [17] Humphrey, P.P.A.; Hartig, P.; Hoyer, D. A proposed new nomenclature for 5-HT receptors. *Trends Pharmacol. Sci.*, **1993**, *14*, 233-236.
- [18] Lubbert, H.; Hoffman, B. J.; Snutch, T. P.; *et al.* cDNA cloning of a serotonin 5-HT_{1C} receptor by electrophysiological assays of mRNA-injected Xenopus oocytes. *Proc. Natl. Acad. Sci. USA*, **1987**, *84*, 4332-4336.
- [19] Julius, D.; MacDermott, A.B.; Axel, R.; *et al.* Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. *Science*, **1988**, *241*, 558-564.
- [20] Yu, L.; Nguyen, H.; Le, H.; *et al.* The mouse 5-HT_{1C} receptor contains eight hydrophobic domains and is X-linked. *Mol. Brain Res.*, **1991**, *11*, 143-149.
- [21] Saltzman, A.G.; Morse, B.; Whitman, M.M.; *et al.* Cloning of the human serotonin 5-HT₂ and 5-HT_{1C} receptor subtypes. *Biochem. Biophys. Res. Comm.*, **1991**, *181*, 1469-1478.
- [22] Canton, H.; Emeson, R.B.; Barker, E.L.; *et al.* Identification, molecular cloning, and distribution of a short variant of the 5-hydroxytryptamine_{2C} receptor produced by alternative splicing. *Mol. Pharmacol.*, **1996**, *50*, 799-807.
- [23] Burns, C.M.; Chu, H.; Rueter, S.M.; *et al.* Regulation of Serotonin-2C receptor G-protein coupling by RNA editing. *Nature*, **1997**, *387*, 303-308.
- [24] Palacios, J.M.; Waeber, C.; Mengod, G.; *et al.* Autoradiography of 5-HT receptors, A critical appraisal. *Neurochem. Int.*, **1991**, *18*, 17-25.
- [25] Radja, F.; Laporte, A.M.; Daval, G.; *et al.* Autoradiography of serotonin receptor subtypes in the central nervous system *Neurochem. Int.*, **1991**, *18*, 1-15.
- [26] Sheldon, P.W.; Aghajanian, G.K. Excitatory responses to serotonin (5-HT) in neurons of the rat piriform cortex, evidence for mediation by 5-HT_{1C} receptors in pyramidal cells and 5-HT₂ receptors in interneurons. *Synapse*, **1991**, *9*, 208-218.
- [27] Rick, C.E.; Stanford, I. M.; Lacey, M.G. Excitation of rat substantia nigra pars reticulata neurons by 5-hydroxytryptamine *in vitro*, evidence for a direct action mediated by 5-hydroxytryptamine_{2C} receptors. *Neuroscience*, **1995**, *69*, 903-913.
- [28] Hoffman, B.J.; Mezey, E. Distribution of Serotonin 5-HT_{1C} receptor mRNA in adult rat brain. *FEBS Lett.*, **1989**, *247*, 453-462.
- [29] Molineaux, S. M.; Jessell, T. M.; Axel, R.; *et al.* 5-HT_{1c} receptor is a prominent serotonin receptor subtype in the central nervous system. *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 6793-6797.
- [30] Sharma, A.; Punhani T.; Fone, K. C. F. Distribution of the 5-hydroxytryptamine_{2C} receptor protein in adult rat brain and spinal cord determined using a receptor-directed antibody, effect of 5,7-dihydroxytryptamine. *Synapse*, **1997**, *27*, 45-56.
- [31] Schlunzen, Saunders-Bush, E.; Burris, K.D.; Knoth, K. Lysergic acid diethylamide and 2,5-dimethoxy-4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. *J. Pharmacol. Exp. Ther.*, **1988**, *246*, 924-928.
- [32] Boess, F. G.; Martin, I.L. Molecular biology of 5-HT receptors. *Neuropharmacology*, **1994**, *33*, 275-317.
- [33] Conn, P.J.; Saunders-Bush, E. Relative efficacies of piperazines at the phosphoinositide hydrolysis-linked serotonergic (5-HT₂ and 5-HT_{1c}) receptors. *J. Pharmacol. Exp. Ther.*, **1987**, *242*, 552-557.
- [34] Kaufman, M.J., Hartig, P.R., Hoffman, B.J. Serotonin 5-HT_{2C} receptor stimulates cyclic GMP formation in choroid plexus. *J. Neurochem.*, **1995**, *64*, 199-205.
- [35] Chang, M.; Zhang, L.; Tam, J.P.; Sanders-Bush, E. Dissecting G protein-coupled receptor signaling pathways with membrane-permeable blocking peptides. Endogenous 5-HT(2C) receptors in choroid plexus epithelial cells. *J. Biol. Chem.*, **2000**, *275*, 7021-7029.
- [36] Sander-Bush, E; Fentress, H; Hazelwood, L. Serotonin 5-HT₂ receptors, molecular and genomic diversity. *Mol. Interv.*, **2003**, *3*, 319-330.
- [37] Price, R.D.; Sanders-Bush, E. RNA editing of the human serotonin 5-HT(2C) receptor delays agonist-stimulated calcium release. *Mol. Pharmacol.*, **2000**, *58*, 859-862.
- [38] Niswender, C. M.; Copeland, S.C.; Herrick-Davis, K.; Emerson, R. B.; Saunders-Bush, E. RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.*, **1999**, *274*, 9472-9478.
- [39] Gurevich, I.; Tamir, H.; Arango, V.; Dwork, A. J.; Mann, J. J.; Schmauss, G. Altered editing of Serotonin 2C receptor pre-mRNA in the prefrontal cortex of depressed suicide victims. *Neuron*, **2002**, *34*, 349-356.
- [40] Lee, G.V.; Jones, E.J. Epilepsy. *Neurobiology of Diseases*, **2000**, *7*, 549-551.
- [41] Cosford, N. D. P.; McDonald, I. A.; Schweiger, E. J. Recent Progress in Antiepileptic Drug Research. *Annu. Rep. Med. Chem.*, **1998**, *33*, 61-70.
- [42] Przegalinski, E.; Baran, L.; Siwanowicz, J. Role of 5-hydroxytryptamine receptor subtypes in the 1-[3-(trifluoromethyl)phenyl] piperazine-induced increase in threshold for maximal electroconvulsions in mice. *Epilepsia*, **1994**, *35*, 889-894.
- [43] Wada, Y.; Shiraishi, J.; Nakamura, M.; Koshino, Y. Role of serotonin receptor subtypes in the development of amygdaloid kindling in rats. *Brain Res.*, **1997**, *747*, 338-342.
- [44] Applegate, C.D.; Tecott, L.H. Global increases in seizure susceptibility in mice lacking 5-HT_{2C} receptors, a behavioral analysis. *Exp. Neurol.*, **1998**, *154*, 522-530.
- [45] Heisler, L. K.; Chu, H. M.; Tecott, L.H. Epilepsy and obesity in serotonin 5-HT_{2C} receptor mutant mice *Ann. N.Y. Acad. Sci. USA*, **1998**, *861*, 74-78.
- [46] Rueter, L.E.; Tecott, L.H.; Blier, P. *In vivo* electrophysiological examination of 5-HT₂ responses in 5-HT_{2C} receptor mutant mice. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **2000**, *361*, 484-491.

- [47] Loscher, W.; Genetic animal models of epilepsy as a unique resource for the evaluation of anticonvulsant drugs. A review. *Methods Find Exp. Clin. Pharmacol.*, **1984**, *6*, 531-547.
- [48] Prendiville, S.; Gale, K.. Anticonvulsant effect of fluoxetine on focally evoked limbic motor seizures in rats. *Epilepsia*, **1993**, *34*, 381-384.
- [49] Yan, Q. S.; Jobe, P. C.; Cheong, J. H.; Ko, K. H.; Daily, J. W. Role of serotonin in the anticonvulsant effect of fluoxetine in genetically epilepsy-prone rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1994**, *350*, 149-152.
- [50] Pasini, A.; Tortorella, A.; Gale, K. The anticonvulsant action of fluoxetine in substantia nigra is dependent upon endogenous serotonin. *Brain Res.*, **1996**, *724*, 84-88.
- [51] Gobert, A.; Rivet, J.; Lejeune, F.; *et al.* Serotonin(2C) receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic pathways, a combined dialysis and electrophysiological analysis in the rat. *Synapse*, **2000**, *36*, 205-221.
- [52] Hutson, P.H.; Barton, C.L.; Jay, M.; *et al.* Activation of mesolimbic dopamine function by phencyclidine is enhanced by 5-HT(2C/2B) receptor antagonists, neurochemical and behavioural studies. *Neuropharmacology*, **2000**, *39*, 2318-2328.
- [53] Huidobro-Toro, J. P.; Valenzuela, C. F.; Harris, R. A. Modulation of GABAA receptor function by G protein-coupled 5-HT_{2C} receptors. *Neuropharmacology*, **1996**, *35*, 1355-1363.
- [54] Favale, E.; Audenino, D.; Cocito, L.; Albano, C. The anticonvulsant effect of citalopram as an indirect evidence of serotonergic impairment in human epileptogenesis. *Seizure*, **2003**, *12*, 316-318.
- [55] Jakus, R.; Graf, M.; Juhasz, G.; Geber, K.; evay, G.; Halasz, P.; Bagdy, G. 5-HT_{2C} receptors inhibit and 5-HT_{1A} receptors activate the generation of spike-wave discharges in a genetic rat model of absence epilepsy. *Exp. Neurol.*, **2003**, *184*, 964-972.
- [56] Upton, N.; Middlemiss, D.; Blackburn, T.; *et al.* Studies on the role of 5-HT_{2C} and 5-HT_{2B} receptors in regulating generalized seizure threshold in rodents. *Eur. J. Pharmacol.*, **1998**, *359*, 33-40.
- [57] Di Matteo, V.; Di Giovanni, G.; Eposito, E. SB-242084, A Selective 5-HT_{2C} Antagonist. *CNS Drug Rev.*, **2000**, *6*, 195-205.
- [58] Bishop, M. J.; Nilsson, B. M. New 5-HT_{2C} Receptor Agonist. *Expert Opinion. Ther. Patent.*, **2003**, *13*, 1691-1705
- [59] Isaac, M. Serotonergic 5-HT_{2C} Receptors as a Potential Therapeutic Target for the Design of Anti-epileptic Drugs. *Drugs Future*, **2001**, *26*, 383-393.
- [60] Bickerdike, M J. 5-HT_{2C} receptor agonists as potential drugs for the treatment of obesity. *Curr. Top. Med Chem.*, **2003**, *3*, 885-897.
- [61] Kimura, Y.; Hatanaka, K.; Naitou, Y.; Maeno, K.; Shimada, I.; Koakutsu, A.; Wanibuchi, F.; Yamaguchi, T. Pharmacological profile of YM348, a novel, potent and orally active 5-HT_{2C} receptor agonist. *Eur. J. Pharmacol.*, **2004**, *483*, 37-43. .
- [62] Im, W. B.; Chio, C.L.; Albert, G. L.; Dinh, D. M. Positive Allosteric Modulator of the Human 5-HT_{2C} Receptor. *Mol. Pharmacol.*, **2003**, *64*, 78-84