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Current status of inverse agonism at serotonin$_{2A}$ (5-HT$_{2A}$) and 5-HT$_{2C}$ receptors

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ABSTRACT

Contemporary receptor theory was developed to account for the existence of constitutive activity, as defined by the presence of receptor signaling in the absence of any ligand. Thus, ligands acting at a constitutively active receptor, can act as agonists, antagonists, and inverse agonists. In vitro studies have also revealed the complexity of ligand/receptor interactions including agonist-directed stimulus trafficking, a finding that has led to multi-active state models of receptor function. Studies with a variety of cell types have established that the serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors also demonstrate constitutive activity and inverse agonism. However, until recently, there has been no evidence to suggest that these receptors also demonstrate constitutive activity and hence reveal inverse agonist properties of ligands in vivo. This paper describes our current knowledge of constitutive activity in vitro and then examines the evidence for constitutive activity in vivo. Both the serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors are involved in a number of physiological and behavioral functions and are the targets for treatment of schizophrenia, anxiety, weight control, Parkinsonism, and other disorders. The existence of constitutive activity at these receptors in vivo, *along with the possibility of inverse agonism*, provides new avenues for drug development.

Contents

1. Introduction

Serotonin (5-HT) is a monoamine neurotransmitter that regulates a wide variety of physiological and behavioral functions, including body temperature, cardiovascular regulation, sleep, pain, mood, body weight, sexual behavior, and cognitive functions (Leysen, 2004; Harvey, Quinn, Liu, Aloyo, & Romano, 2004). This wide variety of functions is mediated by a large collection of receptors, distributed among seven families, all but one of which (the 5-HT$_3$ ligand-gated ion channel) are members of the 7-transmembrane spanning (7-TMS) receptor superfamily, more commonly referred to as G-protein-coupled receptors (GPCRs). The major focus of this paper will be on

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the serotonin 5-HT_{2A} and 5-HT_{2C} receptors. We will describe some of the pharmacological characteristics of these receptors based on in vitro studies, examine some recent evidence for the existence of constitutive activity and inverse agonism in vivo, and describe how the existence of inverse agonist actions at the 5-HT_{2A} and 5-HT_{2C} receptors may provide new avenues for therapeutics.

2. The serotonin 5-hydroxytryptamine$_{2A}$ and 5-hydroxytryptamine$_{2C}$ receptors

The 5-HT_{2A} and 5-HT_{2C} receptor subtypes are widely expressed throughout the brain and the 5-HT_{2A}, but not the 5-HT_{2C}, receptor is also found in the periphery, notably on platelets and smooth muscle cells of the gut vasculature (Leysen, 2004). However, there are still some discrepancies in the identification of the precise cellular localization of 5-HT_{2A} and 5-HT_{2C} receptors in brain. 5-HT_{2A}-like immunoreactivity was reported to be located on pyramidal neurons in rat cortex with a smaller number located on GABAergic interneurons (Willins, Deutch, & Roth, 1997; Jakab & Goldman-Rakic, 1998; Miner, Backstrom, Sanders-Bush, & Sesack, 2003). However, Miner et al. (2003) also noted that 24% of the 5-HT_{2A}-like immunoreactivity was located on presynaptic axons and varicosities that resembled monoamine fibers. 5-HT_{2C}-like immunoreactivity has also been reported on pyramidal cells of frontal cortex and hippocampus with an ~60 to 80% colocalization with 5-HT_{2A}-like immunoreactivity (Alex, 2007). However, the localization of 5-HT_{2C}-like immunoreactivity on interneurons remains problematic. As just two examples of conflicting results, 5-HT_{2C}-like immunoreactivity has been either observed on interneurons in frontal cortex (Liu, Bubar, Lanfranco, Hillman, & Cunningham, 2007) or reported to be absent (Alex, 2007). Thus, there is general agreement that 5-HT_{2A} receptors are located on both pyramidal cells and interneurons, but while 5-HT_{2C} receptors appear to be located on pyramidal cells of cortex their presence on interneurons remains open to question. The possible basis for these discrepant findings had been noted earlier by Willins et al. (1997) who pointed out that "Whatever the reason for the differences obtained the present findings clearly indicate that investigators should be judicious in their choice of epitopes and coupling techniques, and suggest that, when possible, immunolocalization studies should be performed using two or more antibodies directed against the target protein." To which one can only add that it would also be of value to employ an antibody that had been used by other investigators as a positive control.

There is general agreement that 5-HT_{2C}-like immunoreactivity occurs in choroid plexus as well as on interneurons in various subcortical structures including striatum, ventral tegmental area, and substantia nigra, areas that may serve to regulate dopamine release (see review by Alex & Pehek, 2007). It has been suggested that both 5-HT_{2A} and 5-HT_{2C} receptor subtypes are also located on cholinergic (Quirion, Richard, & Dam, 1985), glutamatergic (Aghajanian & Marek, 2000; Lambe, Goldman-Rakic, & Aghajanian, 2000; Hasuo, Matsuoka, & Akasu, 2002), and dopaminergic (Pazos, Probst, & Palacios, 1987; Miner et al., 2003) axon terminals. Activation of these presynaptic receptors could regulate excitability in an extended neuronal circuitry through alteration in the release of glutamate (Ceglia et al., 2004; Muschamp, Regina, Hull, Winter, & Rabin, 2004), acetylcholine (Nair & Gudelsky, 2004), and dopamine (Schmidt, Fadayel, Sullivan, & Taylor, 1992).

The pharmacologic characteristics of the 5-HT_{2A} and 5-HT_{2C} receptors are quite similar owing in part to the high level of amino acid sequence homology between them (≈50% overall and ≈80% within the transmembrane spanning domains). In addition, cellular signaling pathways (e.g. phospholipase C [PLC] and phospholipase A_2 [PLA_2]) regulated by these receptors are similar, although some differences have been reported (Berg, Clarke, Sailstad, Saltzman, & Maayani, 1994; Berg & Clarke, 2001; Berg, Stout, Maayani, & Clarke, 2001). Consequently, identification of the relative involvement of 5-HT_{2A} and 5-HT_{2C} receptors in various behaviors and physiological functions has been difficult due to the relative paucity of receptor-selective ligands especially when such ligands are used in vivo.

3. mRNA editing of serotonin 5-hydroxytryptamine$_{2C}$ receptors

The 5-HT_{2C} receptor is the only 7-TMS receptor to date found to undergo the post-transcriptional process of mRNA editing which generates unique isoforms of proteins in a cell and/or tissue specific manner (Simpson & Emeson, 1996; Smith, Gott, & Hanson, 1997). mRNA transcripts of the rat and human 5-HT_{2C} receptor undergo adenosine-to-inosine editing events at five sites which encompass amino acids 156–160 within the putative second intracellular loop of the encoded human receptor resulting in the production of 14 5-HT_{2C} receptor isoforms (Burns et al., 1997; Niswender, Copeland, Herrick-Davis, Emeson, & Sanders-Bush, 1999). In human brain, the non-edited receptor contains the amino acids isoleucine, asparagine, and isoleucine (INI) at positions 156, 158 and 160, respectively, while two of the principle edited isoforms expressed have valine, serine and valine (VSV) or valine, glycine, and valine (VGV) corresponding to these amino acid positions (156, 158 and 160, respectively). Affinity of 5-HT (and therefore potency to activate PLC) is reduced for VSV or VGV receptor isoforms in comparison with the non-edited INI receptor (Burns et al., 1997; Fitzgerald et al., 1999; Niswender et al., 1999). In addition, it has been suggested that G-protein coupling to the VGV and VSV isoforms is reduced (Burns et al., 1997; Fitzgerald et al., 1999; Niswender et al., 1999).

4. The relationship of serotonin 5-hydroxytryptamine$_{2A}$ and 5-hydroxytryptamine$_{2C}$ receptors to psychiatric disorders

The 5-HT_{2C} and 5-HT_{2A} receptors have been implicated in the etiology and treatment of various psychiatric disorders. Activation of 5-HT_{2C} receptors, with agonists such as mCPP and MK-212, results in feelings of anxiety and panic in humans (Mueller, Murphy, & Sunderland, 1985; Charney, Woods, Goodman, & Heninger, 1987; Lowy & Meltzer, 1988; Kahn & Wetzler, 1991; Klein, Zohar, Geraci, Murphy, & Uhde, 1991; Southwick et al., 1997; Benjamin, Geraci, McCann, Greenberg, & Murphy, 1999; Gatch, 2003) and induces anxiogenic-like behaviors in animals (Kennett, Whitton, Shah, & Curzon, 1989; Benjamin, Lal, & Meyerson, 1990; Rodgers et al., 1992; Shepherd, Grewal, Fletcher, Bill, & Dourish, 1994; Bilkei-Gorzo, Gyertyan, & Levay, 1998; Bagdy, Graf, Anheuer, Modos, & Kantor, 2001; Jones, Duxon, & King, 2002; Martin, Ballard, & Higgins, 2002; Campbell & Merchant, 2003; de Mello Cruz et al., 2005; Millan, 2006; Hackler et al., 2007; Cornelio & Nunes-de-Souza, 2007). 5-HT_{2C} receptor antagonists, on the other hand, can block the anxiogenic-like behavior produced by 5-HT_{2C} receptor agonists (Kennett et al., 1989; Bagdy et al., 2001; Campbell & Merchant, 2003; de Mello Cruz et al., 2005; Cornelio and Nunes-de-Souza, 2007; Hackler et al., 2007) and also are anxiolytic when administered alone (Kennett, Bailey, Piper, & Blackburn, 1995; Kennett et al., 1997; Wood et al., 2001; Wood, 2003; Hackler et al., 2007). Interestingly, anxiogenic behavior induced by acute administration of SSRI antidepressants (e.g. fluoxetine) can be blocked by 5-HT_{2C} receptor antagonists (Bagdy et al., 2001) and with prolonged SSRI administration, 5-HT_{2C} receptors down-regulate with a time–course similar to that associated with improvement of clinical symptoms (Bristow, O'Connor, Watts, Duxon, & Hutson, 2000).

Evidence also suggests that 5-HT_{2C} receptors play a role in schizophrenia (for a review see Meltzer, 1999). RNA-editing efficiency is reduced in the brains of schizophrenic patients (Sodhi, Burnet, Makoff, Kerwin, & Harrison, 2001) and many, but not all (Rauser, Savage, Meltzer, & Roth, 2001), of the atypical antipsychotic drugs have antagonist or inverse agonist properties at the 5-HT_{2C} receptor (Herrick-Davis, Grinde, & Teitler, 2000; Kuoppamaki, Palvimaki, Hietala, & Syvalahti, 1995; Leysen, 2004). 5-HT_{2C} receptor antagonists