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Role for serotonin2A (5-HT2A) and 2C (5-HT2C) receptors in experimental absence seizures

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ABSTRACT

Absence seizures (ASs) are the hallmark of childhood/juvenile absence epilepsy. Monotherapy with first-line anti-absence drugs only controls ASs in 50% of patients, indicating the need for novel therapeutic targets. Since serotonin family-2 receptors (5-HT_2Rs) are known to modulate neuronal activity in the cortico-thalamo-cortical loop, the main network involved in AS generation, we investigated the effect of selective 5-HT_{2A}R and 5-HT_{2C}R ligands on ASs in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a well established polygenic rat model of these non-convulsive seizures. GAERS rats were implanted with fronto-parietal EEG electrodes under general anesthesia, and their ASs were later recorded under freely moving conditions before and after intraperitoneal administration of various 5-HT_{2A}R and 5-HT_{2C}R ligands. The 5-HT_{2A} agonist TCB-2 dose-dependently decreased the total time spent in ASs, an effect that was blocked by the selective 5-HT_{2A} antagonist MDL11,939. Both MDL11,939 and another selective 5-HT_{2A} antagonist (M100,907) increased the length of individual seizures when injected alone. The 5-HT_{2C} agonists lorcaserin and CP-809,101 dose-dependently suppressed ASs, an effect blocked by the selective 5-HT_{2C} antagonist SB 242984. In summary, 5-HT_{2A}Rs and 5-HT_{2C}Rs negatively control the expression of experimental ASs, indicating that selective agonists at these 5-HT_2R subtypes might be potential novel anti-absence drugs.

1. Introduction

Since the original suggestion in the late 1950s' (Bonnycastle et al., 1957), many studies have supported the idea that the serotonin (5-HT) system might be implicated in both focal and generalized epilepsy. In particular, it has been shown that an increase in 5-HT tone is associated with an increased seizure threshold (and/or antiepileptic activity), whilst a reduced seizure threshold follows a decrease in 5-HT levels (reviewed in (Bagdy et al., 2007)). Moreover, many anti-epileptic drugs enhance brain extracellular 5-HT levels and many selective serotonin reuptake inhibitors (SSRIs) show an antiepileptic effect (Bagdy et al., 2007). Despite this large body of evidence, none of the currently available anti-epileptic drugs preferentially targets the 5-HT system, probably because of the lack of selective/specific ligands, the presence of harmful off-target effects and the complexity of the 5-HT receptor (5-HTR) system and its signaling pathways (Hannon and Hoyer, 2008; Stroth and Svenningsson, 2012).

Abbreviations: Serotonin, (5-HT); 5-HT receptor, (5-HTR); Knockout, (KO); Meta-chlorophenylpiperazine, (mCPP); Maximal dentate gyrus activation, (MDA); Absence seizures, (ASs); Genetic absence epilepsy rat from Strasbourg, (GAERS); Spike-and-wave discharges, (SWDs); Intraperitoneally, (i.p.); Two-way analysis of variance, (ANOVA); Wistar Albino Glaxo/Rij, (WAG/Rij); Thalamic reticular nucleus, (NRT); Rapid eye movement, (REM); Cannabinoid, (CB).

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The current classification of 5-HTRs comprises up to 14 subtypes and the generation of selective pharmacological and genetic tools, i.e., knockout (KO) mice, to investigate the contribution of individual receptors is fairly recent (Hannon and Hoyer, 2008). Among the different 5-HTR subtypes (Hoyer et al., 2002), many lines of evidence suggest an involvement of 5-HT_2Rs in seizures (reviewed in (Di Giovanni and De Deurwaerdère, 2016; Guiard and Di Giovanni, 2015; Isaac, 2005; Jakus and Bagdy, 2011)). 5-HT_{2C}R KO mice display spontaneous tonic-clonic seizures which are occasionally lethal (Tecott et al., 1995), and a decreased threshold for various convulsing stimuli, e.g., kindling, pentylenetetrazol (PTZ), electroshock, audiogenic stimuli (Applegate and Tecott, 1998; Heisler et al., 1998). Further evidence of the protective role for 5-HT_{2C}Rs against convulsive seizures comes from experiments using non-selective 5-HT_{2C} agonists which raise the threshold for PTZ- and electroshock-induced seizures (Upton et al., 1998). On the other hand, some 5-HT_{2C}R agonists with different pharmacological profiles, i.e., meta-chlorophenylpiperazine (mCPP) and lorcaserin, but not RO60-0175 (Martin et al., 1998) are able to stop the elongation of the electrically triggered hippocampal maximal dentate gyrus activation (MDA) in a limbic seizure model (Orban et al., 2014). As for 5-HT_{2A}Rs, fewer studies have investigated the role of these receptors in seizures, with most evidence showing that their activation has an antiepileptic effect (Gharedaghi et al., 2014; Guiard and Di Giovanni, 2015).

The evidence of a role for 5-HT_2Rs in generalized non-convulsive seizures is more limited and the interpretation of these studies is hampered by the use of relatively unselective drugs (Bagdy et al., 2007; Di Giovanni and De Deurwaerdère, 2016; Guiard and Di Giovanni, 2015). In the groggy model of absence seizures (ASs) (Tokuda et al., 2007), the 5-$HT_{2A/2C}$ mixed agonist DOI dose-dependently reduces ASs, an effect that is blocked by the non-selective 5-HT_2R antagonist ritanserin (Ohno et al., 2010). In contrast, in the AY-9944 model of atypical ASs, mCPP has no effect, DOI dose-dependently decreases ASs and the moderately selective 5-HT_{2A}R antagonist ketanserin increases ASs in a non-dose-dependent manner (Bercovici et al., 2006). These authors concluded that 5-HT_{2A}Rs were responsible for this effect, although no 5-HT_{2A}R antagonist was tested against the anti-absence action of DOI. As far as typical ASs are concerned, experiments in the Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats, one of the best characterized rat models of these type of non-convulsive seizures (Coenen and Van Luijtelaar, 2003), have found that mCPP decreases ASs via 5-HT_{2C}Rs (Jakus et al., 2003). Moreover, SB-242084 a selective 5-HT_{2C}R antagonist, has no effect on ASs when administered alone, suggesting that 5-HT_{2C}Rs do not play a tonic modulatory role (Jakus and Bagdy, 2011; Jakus et al., 2003). In the other well characterized rat model of typical ASs, the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) (Danober et al., 1998) the contribution of the 5-HT system to ASs has only been partly investigated, probably because of the early negative results obtained with broad-spectrum first-generation 5-HTR agonists and antagonists or following modulation of the 5-HT tone by 5-HT uptake blockers and precursors (Marescaux et al., 1992a, 1992b) (reviewed in (Danober et al., 1998)).

In the present study, we evaluated the effects of pharmacological manipulation of 5-HT_2Rs on typical ASs and the interictal EEG in GAERS using drugs selective for 5-HT_{2A}Rs and 5-HT_{2C}Rs. The potent 5-HT_{2A}R agonist TCB-2 (McLean et al., 2006) was used in combination with the selective 5-HT_{2A}R antagonists MDL11,939 (Dudley et al., 1988) and M100,907 (Table 1) (Kehne et al., 1996). As far as 5-HT_{2C}Rs were concerned, we used CP-809,101, which shows ~1000 fold selectivity for the 5-HT_{2C}R over 5-HT_{2A}R and represents the most selective 5-HT_{2C}R drug currently available (Siuciak et al., 2007), and SB-242084, the most selective 5-HT_{2C}R antagonist synthetized to date (Di Matteo et al., 2000; Kennett et al., 1997) (Table 1). Moreover, the anti-absence action of lorcaserin (Thomsen et al., 2008) was also investigated because, although it has only an approximately 10-fold higher affinity for 5-HT_{2C}Rs compared to 5-HT_{2A}Rs (Table 1), it is the first-in-class 5-HT_{2C}R agonist available for human use (FDA, 2012) and has shown an antiepileptic profile in an animal model of temporal lobe epilepsy (Orban et al., 2014). Our results show that both 5-HT_{2A}Rs and 5-HT_{2C}Rs negatively control the expression of experimental ASs, suggesting that selective agonists at these 5HT_2R subtypes might be potential novel anti-absence drugs.

2. Methods

Male GAERS rats (3–5 months old) were obtained from a colony bred at Cardiff University. Animals were housed in a 12:12 light cycle (lights on at 10.00 p.m. and off at 10.00 a.m.). All animal procedures were approved by the UK Home Office and carried out in accordance with Cardiff University ethical guidelines and in conformity with international law and policies (EU Directive, 2010/63/EU for animal experiments, ARRIVE guidelines and the Basel declaration including the 3R concept). All efforts were made to minimize animal suffering and to reduce the number of animals used (Lidster et al., 2015).

2.1. Surgery and EEG recordings

GAERS underwent chronic electrode implantation under general anesthesia (2% isofluorane). Epidural EEG electrodes (gold plated screws, Svenska Dentorama AB, Sweden) were implanted bilaterally over the frontal cortex, parietal cortex and in the cerebellum, as previously described (Cope et al., 2009). Animals were allowed to recover for at least 5 days. At 10.00 a.m. on the day of the experiment, the animals were placed into individual Plexiglas cages with access to food and water, and connected to a pre-amplifier (0.08 Hz high-pass filter, impedance 10 MΩ) and in turn to an analogue EEG amplifier (4-channel BioAmp, SuperTech Inc., Hungary) (1000 gain, low-pass filter at 500 Hz). The signal was digitized at 1000 Hz with a Cambridge Electronic Design (CED) Micro3 D.130 digitizer using CED Spike2 v7.3.

2.2. Experimental protocol

Once GAERS had been connected to the recording apparatus, they were left undisturbed for 1 h (habituation period). After that, video and EEG recordings commenced and continued for 40 min (control period). If the experiment involved pre-treatment of a 5-$HT_{2A/2C}$ antagonist, the antagonist (or the corresponding vehicle) was intraperitoneally (i.p.) injected 10 min before the end of the control period. At the end of the control period, the animal was injected (i.p.) with the 5-HT drug of interest (or corresponding vehicle) and video and EEG recordings continued for the subsequent 2 h (treatment period). Drug injection order and doses were randomized in an incomplete crossover design and each drug-naïve animal received a maximum of three treatments in increasing dose protocols (vehicle, low dose, high dose) or four treatments when testing one dose of an agonist vs antagonist (e.g. vehicle + vehicle, vehicle + agonist, antagonist + vehicle, antagonist + agonist). The washout period was 5 days.

2.3. ASs detection and quantification

The detection of spike-and-wave discharges (SWDs) was semi-automatic, aided by the SeizureDetect script (kindly provided by Steve Clifford, CED) in Spike2 v7.3 (CED, UK), designed to