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ScienceDirect
Neuroscience Letters 418 (2007) 13–17
Neuroscience Letters
www.elsevier.com/locate/neulet

5-HT2 modulation of AY-9944 induced atypical absence seizures

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Received 30 October 2005; received in revised form 23 February 2007; accepted 26 February 2007

Abstract

We investigated the role of 5-HT_{2A} and 5-HT_{2C} receptors in atypical absence seizures (AAS) induced by trans-1,4-bis[2-chlorobenzylaminomethyl] cyclohexane, dihydrocholoride (AY-9944). The total duration and number and mean duration of the spontaneous bursts of slow spike-and-wave discharges (SSWD) that characterize the AY model were measured using electrocorticographic (ECoG) recordings in freely moving animals. In a randomized counterbalanced dose response design, rats were treated with either the 5-HT_{2A} agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI, 0.5, 1 or 2 mg/kg), the 5-HT_{2C} preferring agonist *m*-chlorophenylpiperazine (*m*CPP, 1, 2, or 4 mg/kg), the 5-HT_{2A} antagonist ketanserin (2.5 or 5 mg/kg), or vehicle. DOI significantly reduced the total duration and number of SSWD. In contrast, *m*CPP had no effect on total duration or number of SSWD. Ketanserin exacerbated the number of SSWD at 2.5 mg/kg but produced mixed results at 5.0 mg/kg. However, none of the treatments affected the mean SSWD duration. These data support the hypothesis that 5HT_{2A} receptors are involved in the pathology of experimental atypical absence seizures.

Keywords: Absence seizures; AY-9944; EEG; Serotonin; DOI; *m*CPP

Atypical absence seizures (AAS) is one of the most malignant epilepsies in children [14] and differs markedly from typical absence epilepsy in EEG findings, ictal behavior, and cognitive and behavioral outcome [11,4,2]. Atypical absence seizures (AAS) are more frequent, prolonged, and refractory to medication than typical absence seizures. The frequency of spike-and-wave discharges (SWD) is slower in atypical absence seizures and the incidence of other seizure types is higher than that seen in typical absence seizures. Both typical and atypical absence epilepsy are characterized by an altered state of consciousness during the ictus; however, while associated behavior during the typical absence seizures consists of complete arrest of activity and immobility, children experiencing atypical absence seizures are not completely immobile during the seizure, often being able to walk and talk during the seizure, albeit with impaired consciousness. In atypical absence seizures, the seizure onset and offset is more gradual than that seen with typical absence seizures and the concordance between behavioral and EEG changes is less exact. Atypical and typical absence epilepsies have the same pharmacological profile [7]. The outcome of atypical absence epilepsy in children is characterized by severe impairments in cognition [9]. In contradistinction, the cognitive and behavioral outcome of typical absence epilepsy often is benign [9].

The clinical manifestation, electrographic, behavioral, and pharmacological characteristics of atypical absence seizures in children is reproducible in the AY-9944 (AY) model, an animal model of atypical absence epilepsy induced in Long Evans hooded rats by treatment with a cholesterol biosynthesis inhibitor, *trans*-1,4-bis[2-cloro-benzylaminomethyl]cycloexane dihydrochloride (AY-9944), during development [6]. The seizures in the AY model display the clinical features of atypical absence epilepsy in terms of ictal EEG and behavior, as well as pharmacological and developmental characteristics in both rats and mice [6].

There is abundant evidence that the thalamocortical circuitry is involved in generating cortical spindles and absence seizures. Intrinsic cortical spindles can be transformed into spike-wave-discharges (SWD), which suggests they share the same neuronal circuitry [8,15]. Thalamocortical circuitry is modulated by

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0304-3940/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2007.02.062

ascending serotonergic pathways via the 5-HT 2A and 2C receptor subtypes [19]. For example, using *in vitro* ferret thalamocortical slices, bath application of serotonin abolishes oscillatory spindling activity [16]. Furthermore, the 5-HT_{2A} agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) reduces thalamocortical spindle activity when given either systemically or intrathalamically. Similarly, administrating the 5-HT_{2A} antagonist ketanserin systemically increases thalamocortical spindles [12]. However, the role of 5-HT_2 receptors has not been extensively studied in absence seizures. For example, Jakus et al. [13] report that the 5-HT_{2C} agonist, *m*-chlorophenylpiperazine (*m*CPP), attenuates SWD in the WAG/Rij genetic model of typical absence epilepsy. However, Marescaux et al. [18] treated GAERS absence seizure rats with ketanserin and concluded that 5-HT_2 receptors do not modulate absence seizures in their model.

The purpose of these experiments is to test the hypothesis that 5-HT_{2A} and 5-HT_{2C} receptors are involved in the pathogenesis of AAS using the AY model of this disorder.

Untimed pregnant Long-Evans hooded rats (Charles River, Quebec, Canada) were housed individually at the Lab Animal Services of The Hospital for Sick Children (Toronto, Ontario, Canada). AY-9944 (*trans*-1,4-bis[2-chloro-benzylaminomethyl]cyclohexane dihydrocholoride) a gift from Wyeth-Ayerst (Philadelphia, PA, USA) was dissolved in distilled water and administered subcutaneously (7.5 mg/kg) to suckling rats every 6 days from postnatal (P) day P2 to P20. At P21, male rats were weaned and housed in pairs until adulthood. This dosing schedule of AY is sufficient to induce chronic atypical absence seizures. Thus, AY was not administered during adulthood. Rats were kept in a controlled environment at a 12-h light–dark cycle with lights on at 06:00 h and *ad lib* access to food and water.

All animal procedures are in accordance with the Animal Care Committee at the Hospital for Sick Children, which conforms to rules and regulations from The Canadian Council on Animal Care and Animals for Research Act (Ottawa, Canada). Within subject experimental designs were utilized to minimize the number of animals used and special care was taken to reduce animal suffering.

Surgical implantation of electrocorticographic (EcoG) electrodes was performed at P55. Animals were administered intraperitoneal (i.p.) atropine methyl bromide (0.5 mg/kg) as a pre-anaesthetic 15 min prior to being given pentobarbital (35 mg/kg, i.p.). Four monopolar electrodes were placed over the frontal (2.2 mm anterior from bregma, 3 mm lateral from midline) and parietal (2.2 mm posterior from bregma, 3 mm midline from midline) regions and secured with four watchmaker screws and dental cement [6]. Daily post-surgical monitoring was done by veterinary technicians for 5 days of recovery.

All ECoG recordings were performed from unrestrained rats in individual Plexiglas chambers (Harvard Apparatus, Holliston, MA) after a 20-min adaptation period. Differential ECoG recordings were made on paper using a Grass Polysomnograph using the following settings: low frequency filter (1 Hz), high frequency filter (70 Hz) and notch-filter (60 Hz) (78D, Grass Instruments, Quincy, MA, U.S.A.) between 10:00 and 14:00 h to prevent circadian confounds [17].

Agonist studies were performed as within subject randomized counterbalanced dose response experiments using 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI, $n=6$) or *m*-chlorophenylpiperazine (*m*CPP, $n=8$) as previously described [12], with some modification. Briefly, drug treatments were delivered i.p. on days 1, 3, 7 and 10 using concentrations of DOI (0, 0.5, 1 and 2 mg/kg) and *m*CPP (0, 1, 2, 4 mg/kg) that were based on pilot work in our laboratory. In all cases, distilled water (delivered i.p.) was used as the vehicle. Rats were monitored for stereotypical behaviour (i.e. wet-dog shakes) that commonly occurred at higher doses of DOI [12,22,21]. Similarly, those receiving *m*CPP were also monitored for aberrant behaviour patterns. For the antagonist experiment, we attempted to use the ketanserin dose response curve of Jakala et al. [12] and Marescaux et al. [18]. Both studies used doses ranging from 0 to 20 mg/kg. However, in our pilot studies we found that doses above 5 mg/kg yielded inconsistent results and aberrant electrographic changes which would not allow for reliable quantification of seizures. Ketanserin experiments were performed as separate randomized counterbalanced experiments using either (0, 2.5 mg/kg, $n=4$) or (0, 5 mg/kg, $n=5$) dosing schedules. This experimental design was opted to prevent any confounds as elicited by previous pilot studies. For each experimental session a 1-h baseline ECoG was taken before, and after drug treatment, with 30 min in between.

Slow-spike-wave discharges (SSWD) were scored only if they were bilaterally synchronous, with amplitude greater than 4 times baseline and frequency of 5–6 Hz as previously described [6]. SSWD were quantified and tabulated to yield total SSWD duration per hour, number of SSWD bursts per hour and mean SSWD duration [6,5]. For sake of simplicity, only the number of SSWD (i.e. frequency) was graphically displayed. To analyze the effect of drug, two-way analysis of variance (ANOVA) was performed followed by two-tailed Student's *t*-tests between the baseline and post-treatment, and between drug post-treatments and vehicle. One-way ANOVA was used to verify the reliability of baselines across treatment schedules and to rule out any carry over effect of drugs. For all statistical analysis, an alpha of $p=0.05$ was used as a measure of significance.

A two-way repeated ANOVA performed with session (vehicle or DOI) as between subject and treatment (baseline or post-treatment) as within subject showed a significant effect of treatment [$F(1,40)=15.50$, $p<0.0005$]. Specifically, DOI produced a significant dose-dependent reduction in the number of SSWD bursts at 1 and 2 mg/kg when compared to baseline and vehicle ($p<0.05$) (Fig. 1). DOI produced a significant dose-dependent attenuation of total SSWD duration [$F(1,40)=15.39$, $p<0.0005$]. Specific comparisons showed a reduction in total SSWD duration at 1 and 2 mg/kg when compared to baseline ($p<0.05$) and at 2 mg/kg when compared to vehicle ($p<0.05$) (data not shown). DOI did not significantly change the mean SSWD duration [$F(1,40)=0.340$, $p>0.05$] Performing one-way ANOVA on baselines shows no significant difference in total duration or number of SSWD across testing days.

*m*CPP failed to produce a significant change in number of SSWD bursts [$F(1,56)=0.049$, $p>0.05$] (Fig. 2). Similarly, there was no significant effect on total SSWD