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Repeated asenapine treatment does not participate in the mild stress induced FosB/ΔFosB expression in the rat hypothalamic paraventricular nucleus neurons

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ABSTRACT

Effect of repeated asenapine (ASE) treatment on FosB/ΔFosB expression was studied in the hypothalamic paraventricular nucleus (PVN) of male rats exposed to chronic mild stress (CMS) for 21 days. Our intention was to find out whether repeated ASE treatment for 14 days may: 1) induce FosB/ΔFosB expression in the PVN; 2) activate selected PVN neuronal phenotypes, synthesizing oxytocin (OXY), vasopressin (AVP), corticoliberin (CRH) or tyrosine hydroxylase (TH); and 3) interfere with the impact of CMS. Control, ASE, CMS, and CMS + ASE treated groups were used. CMS included restraint, social isolation, crowding, swimming, and cold. From the 7th day of CMS, rats received ASE (0.3 mg/kg) or saline (300 μl/rat) subcutaneously, twice a day for 14 days. They were sacrificed on the day 22nd (16–18 h after last treatments). FosB/ΔFosB was visualized with avidin biotin peroxidase complex and OXY, AVP, CRH or TH antibodies by fluorescent dyes. Saline and ASE did not promote FosB/ΔFosB expression in the PVN. CMS and CMS + ASE elicited FosB/ΔFosB-expression in the PVN, whereas, ASE did not augment or attenuate FosB/ΔFosB induction elicited by CMS. FosB/ΔFosB-CRH occurred after CMS and CMS + ASE treatments in the PVN middle sector, while FosB/ΔFosB-AVP and FosB/ΔFosB-OXY after CMS and CMS + ASE treatments in the PVN posterior sector. FosB/ΔFosB-TH colocalization was rare. Larger FosB/ΔFosB profiles, running above the PVN, did not show any colocalizations. The study provides an anatomical/functional knowledge about an unaccented nature of prolonged ASE treatment at the level of PVN and excludes its positive or negative interplay with CMS effect. Data indicate that long-lasting ASE treatment might not act as a stressor acting at the PVN level.

1. Introduction

Recent studies have indicated that acute treatments with antipsychotics (ATs) may have a quite extensive impact in the brain and elicit a distinct pattern of Fos expression even outside of the forebrain areas (Kiss et al., 2010; Rajkumar et al., 2013). Since clinical treatments with ATs have a long-lasting effect their action might be affected by different factors of the external environment. Considerable evidence on the basal clinical and behavioral effects of ATs exists (Findling, 2008; Grundmann et al., 2014). Less is known about the efficacy of these drugs under stress conditions. Even, when such data are accessible, they are most often concentrated only to a limited number of brain structures.

Asenapine is a novel psychopharmacologic agent, belonging to atypical antipsychotic drugs approved for the treatment of schizophrenia (Meltzer et al., 2009; Tait et al., 2009; Howland, 2011). Generally, antipsychotics encompass a wide range of receptor targets. ASE also displays a broad receptor profile showing high affinity (pKi) for numerous receptors, including the serotonin 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT5A, 5-HT6, and 5-HT7 receptors, the adrenergic α1, α2A, α2B, and α2C receptors, the dopamine D1, D2, D3, and D4 receptors, and the histamine H1 and H2 receptors (Huang et al., 2008; Tarazi et al., 2008, 2010; Shahid et al., 2009; Marcus et al., 2010; Franberg et al., 2012; Kusumi et al., 2015). Generally, ASE acts as an antagonist except 5-HT1A receptor where behaving as a partial agonist. Acute administration ASE profoundly exserts its impact mainly in forebrain structures including the striatum, septum, nucleus accumbens, and prefrontal cortex (Majercikova et al., 2014, 2016) and the basal magnocellular nucleus of Meynert (Majercikova and Kiss, 2015). Besides the frontal brain structures, little is known about the impact of the repeated ASE treatment on the other brain structures including PVN a principal central target of stressors (Swanson and Sawchenko, 1980; Aguilera, 2011).

The external milieu ceaselessly influences the living organisms with a wide scale of different stressors. Stress represents a complex response of the organism to stressor challenge detectable at different levels, including motor, sensory, autonomic, and cognitive ones (Tennant, 2002; de Medeiros et al., 2005; Southwick et al., 2005). Hypothalamic-

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pituitary-adrenal (HPA) axis is one of the primary recipients as well as responders to stressors (Cullinan et al., 1995; Ferguson et al., 2008), whereas PVN CRH-, AVP-, and OXY-neurons play a significant role in the integration of multiple sources of afferent inputs and central mediation of the effect of stressors (Jezova et al., 1993; Kiss et al., 1996; Herman et al., 2003; Kiss and Mikkelsen, 2005; Aguilera and Liu, 2012; Jurek et al., 2015). It has been shown that different substance classes, including antidepressants, anxiolytics, and glucocorticoids, may modulate the overall manifestation of the neuronal FosB/ΔFosB expression (Grande et al., 2004; Gago et al., 2011; Garcia-Perez et al., 2012). Although, the FosB/ΔFosB expression in the PVN has been investigated under different conditions including chronic intermittent hypoxia (Bathina et al., 2013; Knight et al., 2013), morphine dependence and withdrawal (Nunez et al., 2010), chronic opiate exposure (Garcia-Perez et al., 2012), surgical stress (Das et al., 2009), acute and repeated cocaine administration (Chocyk et al., 2006), experimental neuropathy (Rouwette et al., 2012), chronic voluntary ethanol intake (Li et al., 2010), and streptozotocin treatment (Zheng et al., 2014), the neuronal identity of activated neurons has not been commonly identified.

Repeated ASE treatment may induce a number of side effects including drowsiness, dizziness, and numbness or tingling of the mouth, restlessness, constipation, dry mouth, sleep problems (insomnia), upset stomach, and weight gain. Many of these side effects might be stressful. According to our knowledge, there are no studies available dealing with the effect of ASE in a linkage with a stressor. Therefore, the present study was designated to evaluate the possible modifications in FosB/ΔFosB expression in the PVN after ASE, CMS, and CMS + ASE treatments. The CMS stressogen effect we previously have qualified (Majercikova et al., 2014) as a light stressor enabling to mimic light stressor effects occurring in the human everyday life. The aim of the present study was to find out whether repeated ASE treatment for 14 days may: 1) promote FosB/ΔFosB expression in the PVN, 2) activate neuronal phenotypes, including OXY, AVP, CRH or TH present in the PVN, and 3) interfere with the CMS preconditioning, i.e. whether long-lasting CMS might be modified by ASE or CMS might alter the central impact of ASE treatment. Dual immunohistochemistry was employed to identify the anatomical aspect of the FosB/ΔFosB-labeled profiles within the PVN different subdivisions. The FosB/ΔFosB profiles counting was performed on photomicrographs captured under combined light and fluorescent microscopic illuminations.

2. Experimental procedures

2.1. Animals

Adult male Wistar rats ($n = 28$, Charles River, Germany) weighing 240–260 g were used. They were housed two per cage in a room with controlled temperature (22 ± 1 °C), light (12-hour light/dark cycle with lights on at 06:00 a.m.), and humidity (55 ± 10%). Animals were provided with a regular rat chow (dry pellets) and tap water ad libitum. Principles of the laboratory animal care and the experimental procedures used were approved by the Animal Care Committee of the Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Slovak Republic. The investigation was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. An effort was made to minimize the number of animals used and their suffering.

2.2. Stress model

The rats were divided into 4 groups: control + vehicle (CON, $n = 6$), control + asenapine (ASE, n = 6), CMS + vehicle (CMS, $n = 8$), and CMS + asenapine (CMS + ASE, n = 8). The CMS consisted of a combination of different stressors (Majercikova et al., 2014, 2016), including: restraint (RE, animals were placed into plastic restrainers), social isolation (SI, animals were kept individually in cages), crowding (CR, animals from two cages were placed into one cage), cold (CO, animals with cages were placed into a cold room at 4 °C), and swimming (SW, animals were put into 45 cm high × 20 cm wide glass cylinders, filled with normal 25 ± 1 °C water up to 36 cm of the height). The animals received one stress challenge per day in the following sequence and duration of exposure: RE (from 09:00 to 09:30 a.m.) – SI (overnight) – CR (overnight) – RE (from 12:00 a.m. to 00.30 p.m.) – CO (from 09:00 to 09:30 a.m.) – SI (overnight) – CO (from 12:00 a.m. to 00.30 p.m.) – RE (from 12:00 a.m. to 00.45 p.m.) – CO (from 09:00 to 09:45 p.m.) – SI (overnight) - RE (from 12:00 a.m. to 00.45 p.m.) – SW (from 09:00 to 09:15 a.m., the rats were exchanged in 15 min intervals) – SW (from 09:00 to 09:05 a.m., the rats were exchanged in 5 min intervals) – SI (overnight) – RE (from 12:00 a.m. to 00:30 p.m.) – CO (09:00 to 09:45 a.m.) – SE (overnight) – RE (12:00 a.m. to 01:00 p.m.) – CR (overnight) – CO (12:00 a.m. to 01:00 p.m.) – SI (overnight). In order to minimize the stressors predictability, the particular stressor was applied each day at different time.

The CMS and CMS + ASE animals were exposed to the stressor for 21 days. From the 7th day of the stress, ASE and CMS + ASE groups started to be injected with ASE (0.3 mg/kg b.w. subcutaneously, Sigma St. Louis MO, A7861, dissolved in saline) and CON and CMS ones with vehicle (saline subcutaneously, 300 μl/rat), respectively. Saline or ASE was applied twice a day (between 06:00 and 07:00 a.m., and 05:00 and 06:00 p.m.). On the 22nd day, i.e. 16–18 h after the last treatments, the animals were sacrificed by a transcardial perfusion with fixative. The ASE dose (0.3 mg/kg b.w.) used was selected based on the literature (Tarazi et al., 2008; Marston et al., 2011), where different doses of ASE have been compared in parallel manner. The effect of ASE dose selected was first monitored in a pilot study and then used in experimental studies (Majercikova et al., 2014, 2016; Majercikova and Kiss, 2015, 2016).

2.3. The hypothalamic paraventricular nucleus

The PVN location was identified based on the rat brain atlas (Paxinos and Watson, 2007). In the present study, three PVN sectors, including anterior, middle, and posterior ones, were investigated. The anterior sector included anterior magnocellular (am) and anterior parvocellular (ap); the middle sector dorsal parvocellular (dp), medial parvocellular (mp), and posterior magnocellular (pm); and posterior sector mp and lateral parvocellular (lp), subdivisions (Fig. 1) (Swanson et al., 1981). The spatial boundaries of the PVN subnuclei were assigned based on the mapping of vasopressin and oxytocin neurons within the PVN (Hou-Yu et al., 1986). The single FosB/ΔFosB profiles counting was

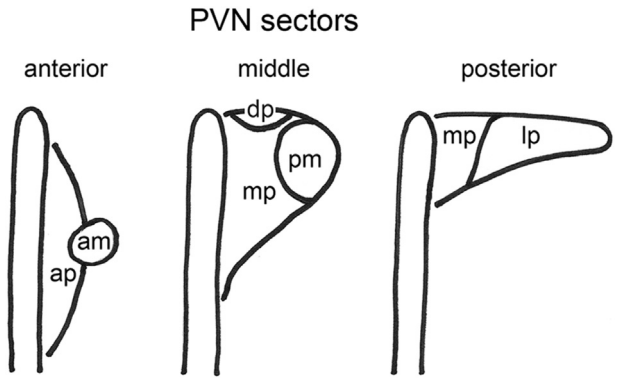

Fig. 1. Schematic illustration of the PVN three sectors (anterior, middle, and posterior) with their subdivisions investigated. The PVN anterior sector contains anterior magnocellular (am) and anterior parvocellular (ap) subdivisions; the middle sector dorsal parvocelluar (dp), medial parvocellular (mp), and posterior magnocelluar (pm) subdivisions; and the posterior sector medial parvocellular (mp) and lateral parvocellular (lp) subdivisions.

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