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6-HYDROXY-L-NICOTINE EFFECTS ON OPEN FIELD ACTIVITY IN THE RAT: IMPLICATIONS FOR A MODEL OF ANXIETY WITH CHLORISONDAMINE

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Abstract
6-Hydroxy-L-nicotine (6HLN) is a nicotine metabolite resulted from nicotine degradation within Arthrobacter nicotinovorans with positive effects on spatial memory and oxidative stress damage. Recently, our group demonstrated that 6HLN could act as a anxiolytic and as an antidepressant agent in chlorisondamine (CHL) rat model. Based on our previous results, in the present study, we hypothesized that 6HLN exhibited anxiolytic effects as assessed by open field test in the CHL rat model. CHL, a neuronal nicotinic ganglionic blocker, when injected into the peritoneal cavity to abolish sympathetic and parasympathetic nerve activity and also, blocks behavioral responses to nicotine for several weeks or months in rats. The blocking of the ion channel(s) prevents nicotine from exerting its rewarding effects on the central nervous system (CNS). The aim of this study was to evaluate the anxiolytic effects of nicotine (0.3 mg/kg) and 6HLN (0.3 mg/kg) using a chlorisondamine (CHL) rat model. The anxiolytic effect was evaluated by open field test. Both nicotine and 6HLN improved cognition related behaviors in anxiety effectively induced by CHL in the laboratory rats.

Keywords: 6-hydroxy-L-nicotine, Alzheimer, anxiety.

1. INTRODUCTION

Dementia encompasses a range of neurological disorders characterised by memory loss and cognitive impairment. Alzheimer's disease (AD) is the most common form of dementia, accounting for 50–70% of cases. The most common early symptom of dementia is difficulty in remembering recent events. As the disorder develops, a wide range of other symptoms can emerge, such as disorientation, mood swings, confusion, more serious memory loss, behavioural changes, difficulties in speaking and swallowing, and problems with walking (Schenk et al., 1998). Progressive accumulation of disability, with deterioration in multiple cognitive domains, interferes with daily functioning, including social and professional functioning. Thus, dementia substantially affects the daily lives of patients, their families, and wider society. Increasing age is the most important risk factor for AD and other dementias, and as life expectancy increases and demographic ageing occurs in populations around the world, the number of people with dementia is expected to increase (American Psychiatric, 1984; Schenk et al., 1998). In 2015, almost 47 million people worldwide were estimated to be affected by dementia, and the numbers are expected to reach 75 million by 2030, and 131 million by 2050, with the greatest increase expected in low-income and middle-income countries. In 2012 and 2015, the World Health Organization (WHO) presented

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reports in which it acknowledged this trend - sometimes described in terms of a fast-growing epidemic - and concluded that AD and other dementias should be regarded as a global public health priority (Schenk et al., 1998; Singh et al., 2004). These patients often exhibit psychiatric symptoms along with cognitive decline. Emotional symptoms of anxiety and phobia contribute significantly to the clinical profile in mild cognitive impairment (MCI) and AD. Emotional behavior critically depends on the amygdala, a region of the temporal lobe that is affected by amyloid-beta peptide (Aβ) and neurofibrillary tangle pathology at early stages of AD (Singh et al., 2004). The current symptomatic treatment of patients with mild-to-moderate AD is based on drugs such as donepezil, rivastigmine, galantamine and memantine which are associated with side effects. These drugs are able to reduce the signs of the disease but have not the potential to treat it. There is currently a high demand for natural therapies to treat AD and reduce the side effects of drugs used in the clinic (Hritcu et al., 2015; Ionita et al., 2017). It has been demonstrated that nicotine exhibit anxiolytic effects at low doses in the rat social interaction test, while high doses induced anxiogenesis (Hritcu et al., 2015). Moreover, in the nicotinic acetylcholine receptor knockout mice, an increased anxiety like behavior in the Elevated Plus Maze was observed (Ionita et al., 2017). 6-Hydroxy-L-nicotine (6HLN) is a nicotine metabolite resulted from nicotine degradation within Arthrobacter nicotinovorans with positive effects on spatial memory and oxidative stress damage (Castilla-Ortega et al., 2014; Ionita R et al., 2017. Recently, our group demonstrated that 6HLN could act as a anxiolytic and as an antidepressant agent in chlorisondamine (CHL) rat model (Castilla-Ortega et al., 2014). In the light of these results, we suggested that 6HLN could represent a viable therapeutic alternative to improve cognitive deficits and reducing oxidative damage in AD (Schenk et al., 1998; Castilla-Ortega et al., 2014. Based on our previous results, in the present study, we hypothesized that 6HLN exhibited anxiolytic effects as assessed by open field test in the CHL rat model. CHL, a neuronal nicotinic ganglionic blocker, when injected into the peritoneal cavity to abolish sympathetic and parasympathetic nerve activity and also, blocks behavioral responses to nicotine for several weeks or months in rats (Schenk et al., 1998). The blocking of the ion channel(s) prevents nicotine from exerting its rewarding effects on the central nervous system (CNS)) (Hritcu et al., 2015).

2. MATERIALS AND METHODS

Animals. The study used 30 male Wistar rats (5-6-month-old) weighing 250 ± 50 h at the start of the experiment were used. The animals were housed in a temperature and light-controlled room (22º C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. The rats were divided into 6 groups (5 animals per group): (1) control group received saline treatment (0.9% NaCl); (2) nicotine (Nic)-alone-treated group; (3) 6-hydroxy-L-nicotine (6HLN)-alone-treated group; (4) chlorisondamine (CHL)-alone-treated group; (5) chlorisondamine (CHL)-treated group received nicotine treatment (CHL+Nic) and (6) chlorisondamine-treated group received 6HLN treatment (CHL+6HLN). Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare from Romania and all procedures were in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. This study was approved by the Committee on the Ethics of Animal Experiments of the Alexandru Ioan Cuza University of Iasi (permit number: 2198) and also, efforts were made to minimize animal suffering and to reduce the number of animals used.

Open Field Test (OFT). Motor behaviors in the OFT were studied in an opaque open field (100 cm × 100 cm × 40 cm) using a floor marked with lines forming 20 cm × 20 cm squares, as previously

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described (Castilla-Ortega et al., 2014). A central square (20 cm × 20 cm) was drawn in the middle of the open field. The field was illuminated using a ceiling halogen lamp regulated to 350 lx at the center of the field. Rats were placed into the center of the open field and allowed exploring the apparatus for 5 min. After the 5 min. test, rats were returned in their home cages and the open field was cleaned with cotton and 10% ethanol solution between subjects. Locomotor activity (the number of lines that the animal crossed with all four paws), the time spent in the center of the field were assessed, number of defects, and the number of urination. The open field floor was cleaned with cotton and 10% ethanol solution between subjects.

Drug administration.

Nicotine and 6HLN were acute administrated, daily, for 7 consecutive days, at a dose of 0.3 mg/kg b.w., i.p. and also with 30 min before the behavioral tests. Chlorisondamine (10 mg/kg, b.w., i.p.) was administrated individually (CHL) or in the combination with nicotine (CHL+Nic) or with 6-hydroxy-L-nicotine (CHL+6HLN), 24 hours before the behavioral testing. Control animals received i.p. an equal volume of sterile saline (1 ml/kg b.w).

Statistical analysis.

The animal's behavioral activities in open field test tasks were statistically analyzed by one-way analysis of variance (ANOVA) using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA following by Tukey's post hoc test. All results are expressed as mean ± standard errors of the mean (S.E.M). F values for which $p < 0.05$ were regarded as statistically significant.

3. RESULTS AND DISCUSSIONS

Anxiety in open filed test.

As can be seen in the Fig. 1, one-way ANOVA revealed significant overall differences between groups ($F(6, 28)=7.54$, $p<0.001$). Increases in time spent on the center area of the open field can be interpreted as an anxiolytic-like effect. In this experiment, CHL by itself significantly affect the amount of the percentage of the time spent in the center of the apparatus as compared to control group ($p<0.001$), whereas Nic and 6HLN significantly increased ($p<0.01$) the percentage of time spent on the center as compared to control group. Nic and 6HLN also increased the total number of crossing square in the open field test, while CHL significantly decreased the number of crossing, as a measure of anxiety-like potential. As can be seen in the Fig. 2, one-way ANOVA revealed non-significant overall differences between groups ($F(6,28)=1.03$, $p>0.05$). In the CHL-treated rats, both Nic and 6HLN, but especially 6HLN, significantly improved the percentage of time spent in the center ($p<0.001$) and also increased the total number of crossing ($p<0.001$) as compared to CHL group, without affecting locomotor activity as assessed by the number of crossing. In the present study, CHL-treated rats exhibited anxiogenic behavior, as evidenced by the fact that rats has a frequent defecation (Fig. 3). CHL-treated rats have a higher number of urination. Defecation correlated significantly only with urination. The hypothesis that defecation and ambulation are inversely related was not confirmed. Emotionality, in the rat, is best defined by amount of defecation in the open field. Our results would agree with those of André Ramos et al and (Hritcu et al., 2015) that this preference is likely to reflect an aversion towards the center caused by fear or anxiety: significantly more anxiety-related behavior was observed on the center (freezing, immobility, defaecation, urination). However, the acute administration of Nic and 6HLN, removes the effects of CHL, acting as anxiolytic pharmacological agents. Consequently, the improvement of behavioral scores as a result of Nic and 6HLN injection is not attributed to increasing of the locomotor activity.

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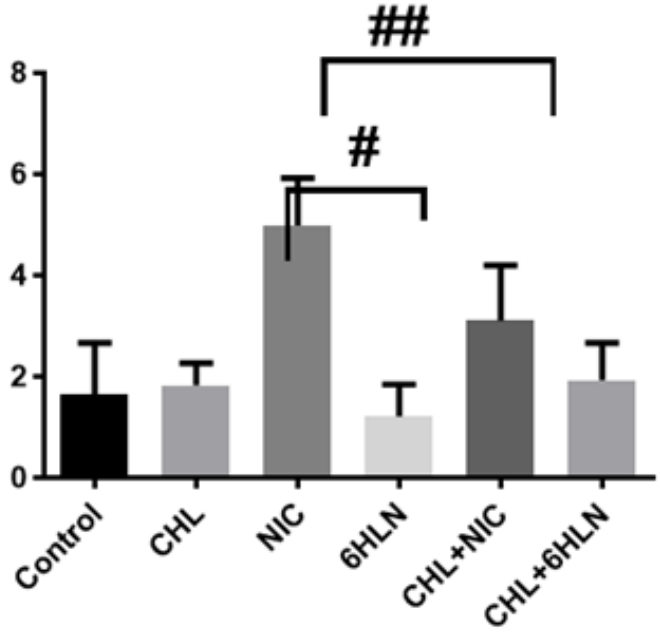

Fig. 1. Effects of the nicotine (Nic, 0.3 mg/kg) and 6-hydroxy-L-nicotine (6HLN, 0.3 mg/kg) in the open field test on the percentage of the time spent in the center. Values are means ± S.E.M. (n = 5 animals per group). For Tuckey's post hoc analyses: ##CHL vs. CHL+Nic: p<0.001 and ##CHL vs. CHL+6HLN: p<0.001 (a) and ##CHL vs. CHL+Nic: p<0.001 and ###CHL vs. CHL+6HLN: p<0.0001

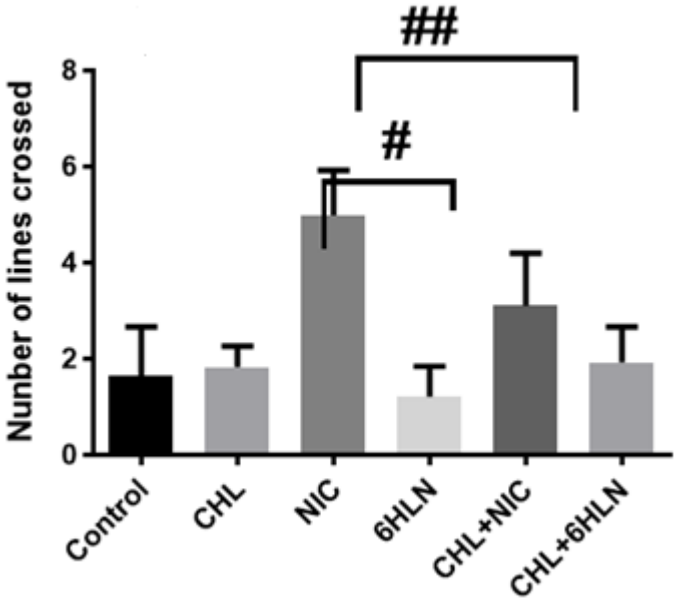

Fig. 2. Effects of the nicotine (Nic, 0.3 mg/kg) and 6-hydroxy-L-nicotine (6HLN, 0.3 mg/kg) in the open field test on the number of crossing. Values are means ± S.E.M. (n = 5 animals per group). For Tuckey's post hoc analyses: ##CHL vs. CHL+Nic: p<0.0001 and ##CHL vs. CHL+6HLN: p<0.0001 (a) and ##CHL vs. CHL+Nic: p<0.0001 and ###CHL vs. CHL+6HLN: p<0.0001

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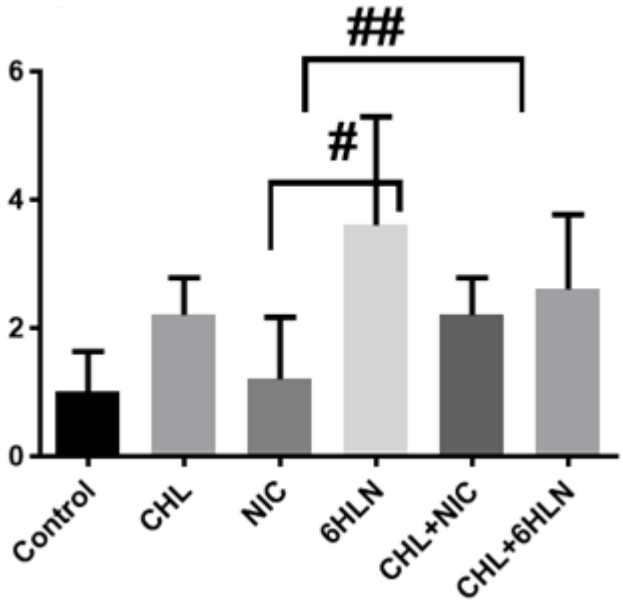

Fig. 3. Effects of the nicotine (Nic, 0.3 mg/kg) and 6-hydroxy-L-nicotine (6HLN, 0.3 mg/kg) on the number of urination (a) and number of defecation (b) in the chlorisondamine (CHL)-treated rats during the 5 min period in the open field test. Values are means ± S.E.M. (n = 6 animals per group). For Turkey's post hoc analyses - #CHL vs. CHL+Nic: p<0.01 and ##CHL vs. CHL+6HLN: p<0.0001 (a).

4. CONCLUSIONS

The open field test was performed to evaluate locomotion and anxiety-like behaviors; stress affected the time spent in the center and periphery. Reduced time spent in the OFT center is used to measure the anxiety-like behavior. The present results suggest that both nicotine and 6-hydroxy-L-nicotine act as anxiolytic agents in the chlorisondamine rat model. The observed effects could be mediated by the nicotinic acetylcholine receptors.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF NTEREST

The authors declare that they have no potential conflicts of interest to disclose.

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