ORIGINAL ARTICLE

6-HYDROXY-L-NICOTINE EFFECTS ON ANXIETY AND DEPRESSION IN A RAT MODEL OF CHLORISONDAMINE

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Abstract

6-hydroxy-L-nicotine (6HLN), a nicotine derivative from nicotine degradation by *Arthrobacter nicotinovorans* pAO1 strain, was found to improve behavioural deficits and to reverse oxidative stress in the rat brain. We evaluated the anxiolytic and antidepressant effects of nicotine (0.3 mg/kg b.w.) and 6HLN (0.3 mg/kg b.w.) using a chlorisondamine (CHL) rat model. The anxiolytic effect was evaluated by elevated plus maze test, while the antidepressant effect was investigated using the forced swimming test. Both nicotine and 6HLN improved cognition related behaviours in anxiety and depression, effectively induced by CHL in the laboratory rats.

Rezumat

Studii recente au demonstrat că 6-hidroxi-L-nicotina (6HLN), un derivat al nicotinei rezultat prin degradarea acesteia de către *Arthrobacter nicotinovorans* pAO1, a îmbunătățit deficitele comportamentale și activitatea sistemului antioxidant din creierul de șobolan. Acest studiu a fost efectuat în vederea evaluării efectelor anxiolitice și antidepresive ale nicotinei (0,3 mg/kg corp) și 6HLN (0,3 mg/kg corp), utilizându-se un model animal cu clorizondamină (CHL). Efectul anxiolitic a fost evaluat cu ajutorul testului labirintului în cruce, în timp ce efectul antidepresiv a fost evaluat cu ajutorul testului înotului forțat. Nicotina și 6HLN au îmbunătățit răspunsurile comportamentale ale șobolanilor tratați cu CHL.

Keywords: nicotine, 6-hydroxy-l-nicotine, chlorisondamine, anxiety, depression, Alzheimer's disease

Introduction

Alzheimer's disease (AD) is a degenerative brain disease and the most common cause of dementia, characterized by decline in memory, language, problem-solving and other cognitive skills that affect a person's ability to perform everyday activities [1, 11].
It is estimated that 24 million people worldwide have dementia of the Alzheimer type and that AD alone contributes with 11.2 % of years lived with disability in people aged 60 years and older; more than other age-related diseases (stroke, musculo-skeletal disorders, cardiovascular diseases and all forms of cancer) [6]. 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 behaviour 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 [3]. 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 [2]. It has been demonstrated that nicotine exhibit anxiolytic effects at low doses in the rat social interaction test, while high doses induced anxiogenesis [10]. Moreover, in the nicotinic acetylcholine receptor knockout mice, an increased anxiety like behaviour in the elevated plus maze test was observed [10].
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 [2, 4, 5]. Recently, our group demonstrated that 6HLN could act as a memory-enhancer and as an antioxidant agent in both scopolamine (Sco) and chlorisondamine (CHL) rat model [2, 5]. In the light of these results, we suggested that 6HLN could represent a viable therapeutic alternative to improve cognitive deficits and to reduce oxidative damage in AD [2, 5]. Based on our previous results, in the present study, we hypothesized that 6HLN exhibited anxiolytic and

antidepressant effects as assessed by elevated plus-maze and forced swimming tests 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 behavioural 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) [9].

Materials and Methods

Chemicals. Nicotine, 6-hydroxy-L-nicotine, chlorisondamine, diazepam, tramadol were purchased from Sigma-Aldrich, Germany.

Animals. The study used 30 male Wistar rats (5 - 6 month-old) weighing 250 ± 50 g at the start of the experiment. The animals were housed in a temperature and light-controlled room (22ºC, 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).In addition, there were two more groups as referred to diazepam group (DZP) and tramadol group (TRM) used as positive controls within the elevated plus maze and forced swimming tests. 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 the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 regarding 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 Iași, Romania, (permit number: 2198) and also, efforts were made to minimize animal suffering and to reduce the number of animals used.

Elevated plus-maze (EPM). Behaviour in the EPM was also utilized to assess exploration, anxiety, and motor behaviour. The elevated plus-maze was made of gray Plexiglas and consists of four arms, 49 cm long and 10 cm wide, elevated 50 cm above the ground. Two arms were enclosed by 30 cm high walls and the other two arms were exposed. It was mounted on a Plexiglas base raised 39 cm above the floor. Light levels on the open and enclosed arms were similar. A video camera was mounted on the ceiling above the apparatus and the experiments were taped for later behavioural evaluation. At the beginning of the experiment, the rat was placed in the centre of the maze and the following variables were scored: (1) the time spent in the open arms and in the enclosed arms; (2) the number of entries to any of the four arms. An arm entry was defined as the entry of all four feet of the animal into one arm. The test lasted 5 min and the apparatus was thoroughly cleaned after removal of the rat with cotton and 10 % ethanol solution [12].

The forced swimming test (FST). In the FST, rats were introduced into transparent cylindrical plastic tanks (height = 59 cm, internal diameter = 30 cm) containing water to a level of 25 cm (26 ± 1ºC). Water was always changed for each rat. Rats are exposed to a 15 min pre-test swim period and followed the next day with a 6 min test swim session. Both the swim sessions are conducted between 12.00 - 18.00 h. After each swim session, the rats are removed from the water, dried with towels and placed in the warm enclosure for 20 min and then returned to their home cages. During the single exposure to forced swimming (6 min), the behaviour of the rat was recorded on videotape. The following two types of behaviour were distinguished and measured with a stopwatch by one experimenter: (1) immobility (time spent floating with the minimal movements to keep the head above the water); and (2) swimming (time spent with active swimming movements) [7].

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 behavioural 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 behavioural testing. Control animals received i.p. an equal volume of sterile saline (1 mL/kg b.w.).

Statistical analysis. The animal's behavioural activities in the elevated plus maze and the forced swimming test tasks were statistically analysed 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.

Results and Discussion

Anxiety in elevated plus maze task. As can be seen in the Figure 1a, one-way ANOVA revealed significant overall differences between groups ($F(6, 28) = 7.54$, $p < 0.001$). Increases in the time spent on the open arms can be interpreted as an anxiolytic-like effect.

In this experiment, CHL by itself significantly affects the amount of the percentage of the time spent in the open arms of the apparatus as compared to the control group ($p < 0.001$), whereas Nic and 6HLN significantly increased ($p < 0.01$) the percentage of time spent on the open arms as compared to control group. As can be seen in the Figure 1b, one-way ANOVA revealed significant overall differences between groups ($F(6, 28) = 17.72$, $p < 0.001$). Nic and 6HLN also increased the total number of entries in the open arms, while CHL significantly decreased the number of entries in the open arms, as a measure of anxiety-like potential. As can be seen in the Figure 1c, one-way ANOVA revealed non-significant overall differences between groups ($F(6, 63) = 1.03$, $p > 0.05$). In the CHL-treated rats, both Nic and 6HLN, but especially 6HLN, significantly improved the percentage of time spent in the open arms ($p < 0.001$) and also increased the total number of entries in the open arms ($p < 0.001$) 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 behaviour, as evidenced by the fact that rats prefer the enclosed arms of the EPM to the open arms. CHL-treated rats spent a significantly greater amount of time in the closed arms and entered them more frequently than the open arms. Our results would agree with those of Sandbak *et al.* [8] that this preference is likely to reflect an aversion towards the open arms caused by fear or anxiety: significantly more anxiety-related behaviour was observed on the open arms (freezing, immobility, defaecation). However, the acute administration of Nic and 6HLN, removes the effects of CHL, acting as anxiolytic pharmacological agents. Consequently, the improvement of behavioural scores as a result of Nic and 6HLN injection is not attributed to increasing the locomotor activity.

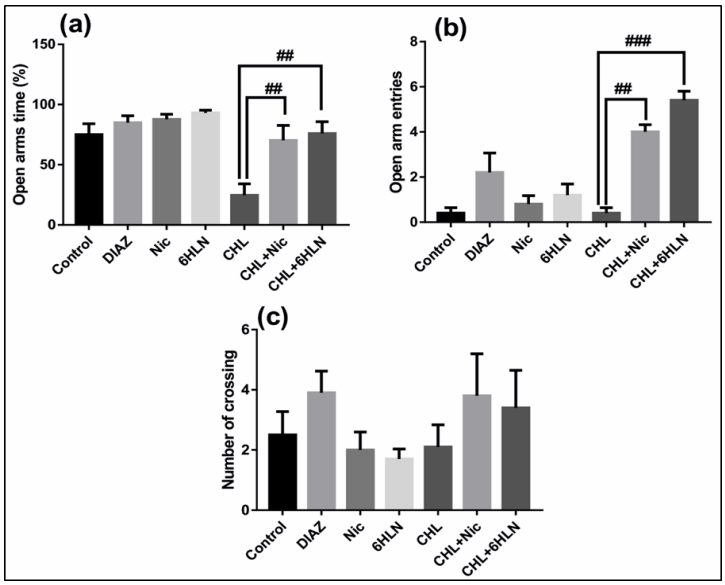

Figure 1.

Effects of the nicotine (Nic, 0.3 mg/kg b.w.) and 6-hydroxy-L-nicotine (6HLN, 0.3 mg/kg b.w.) in the elevated plus-maze test on the percentage of the time spent in the open arms (a) and on the number of open-arm entries in the chlorisondamine (CHL, 10 mg/kg b.w.) - treated rats. 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: $p < 0.00001$ (b). Diazepam group (DZP) was used as positive control.

Depression in forced swimming test. The pharmacological effects of both Nic and 6HLN on CHL-induced memory impairment associated with depression were investigated, and also their possible mechanism of action underlying these effects. As can be seen in Figure 2, one-way ANOVA revealed significant differences between groups for the swimming time ($F (6, 28) = 8.44$, $p < 0.0001$) (a) and for the immobility time ($F (6, 28) = 3.38$, $p < 0.01$) (b). The study demonstrates that acute nicotine and 6HLN administration, but especially 6HLN, induced an increase in the swimming activity and a reduction of the immobility ($p < 0.01$) as compared to the control group. Consequently, administration of CHL caused depressive-like effects. Both Nic and 6HLN administration attenuated depressive-like response in the CHL rat model as compared to CHL-treated rats. Our results are in line with those of Vázquez-Palacios *et al.* [13] suggesting that nicotine administered acutely, sub-chronically, or chronically was found to decrease immobility in adult male Wistar rats, as a measure of its antidepressant effects.

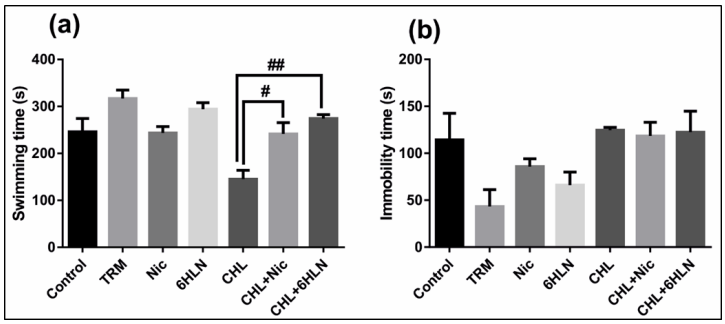

Figure 2.

Effects of the nicotine (Nic, 0.3 mg/kg b.w.) and 6-hydroxy-L-nicotine (6HLN, 0.3 mg/kg b.w.) on swimming time (a) and immobility time (b) in the chlorisondamine (CHL) - treated rats during the 6 min period of the forced swimming 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). Tramadol group (TRM) was used as positive control.

Conclusions

The present results suggest that both nicotine and 6-hydroxy-L-nicotine act as anxiolytic and anti-depressant agents in the chlorisondamine rat model. The observed effects could be mediated by the nicotinic acetylcholine receptors.

Acknowledgement

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Conflict of interest

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

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