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Effects of varenicline on alpha4-containing nicotinic acetylcholine receptor expression and cognitive performance in mice

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ABSTRACT

Nicotinic acetylcholine receptor (nAChR) subtypes containing the α_4 subunit, particularly $\alpha_4\beta_2$ nAChRs, play an important role in cognitive functioning. The impact of the smoking cessation aid varenicline, a selective partial $\alpha_4\beta_2$ nAChR agonist, on (1) changes of central protein and mRNA expression of this receptor and (2) on memory deficits in a mouse model of cognitive impairment was investigated.

Protein and mRNA expression of both the α_4 and β_2 receptor subunits in mouse brain endothelial and hippocampal cells as well as hippocampus and neocortex tissues were determined by western blot and realtime PCR, respectively. The β_2 antibody showed low specificity, though. Tissues were examined following a 2-week oral treatment with various doses of varenicline (0.01, 0.1, 1, 3 mg/kg/day) or vehicle. In addition, episodic memory of mice was assessed following this treatment with an object recognition task using (1) normal mice and (2) animals with anticholinergic-induced memory impairment (i.p. injection of 0.5 mg/kg scopolamine).

Varenicline dose-dependently increased protein expression of both the α_4 and β_2 subunit in cell cultures and brain tissues, respectively, but had no effect on mRNA expression of both subunits. Scopolamine injection induced a significant reduction of object memory in vehicle-treated mice. By contrast, cognitive performance was not altered by scopolamine in varenicline-treated mice.

In conclusion, a 2-week oral treatment with varenicline prevented memory impairment in the scopolamine mouse model. In parallel, protein, but not mRNA expression was upregulated, suggesting a posttranscriptional mechanism. Our findings suggest a beneficial effect of varenicline on cognitive dysfunction.

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1. Introduction

The cholinergic system is long known to play a key role in cognitive functioning in health and disease. In particular, stimulation of nicotinic acetylcholine receptors (nAChRs) consistently improved cognition in experimental animals and humans in multiple studies (Kenney and Gould, 2008; Heishman et al., 2010). Subtypes of nAChRs may thus represent targets for therapeutics for cognitive deficiency in a variety of conditions such as schizophrenia, late-life depression, Alzheimer's disease (AD), attention deficit-hyperactivity disorder (ADHD), and age-associated memory decline (Grupe et al., 2014; Smith et al., 2006; Zurkovsky et al., 2013; Newhouse et al., 2001; Potter et al., 2006; Dunbar et al., 2011). There is substantial evidence linking specifically α_7 and $\alpha_4\beta_2$ nAChRs to cognitive function (Hurst et al., 2013). Both receptor subtypes are most densely expressed in the dentate gyrus and the CA1 region of the hippocampus, areas that are critical to the processing of new information and the formation of memory. In these brain regions, the two mentioned nAChR subtypes are located both pre- and postsynaptically suggesting roles in calcium gating and

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synaptic plasticity (Levin et al., 2006).

The neuronal $\alpha_4\beta_2$ nAChR is of particular interest as a therapeutic target since it shows a high affinity for nicotine and desensitizes slowly. In a study in both rats and mice, application of the selective $\alpha_4\beta_2$ nAChR agonist RJR-2403 mediated a significant memory improvement in a passive avoidance paradigm (Lippiello et al., 1996). In AD and mild cognitive impairment as its prodromal state, there is a marked correlation of cognitive deficits with loss of $\alpha_4\beta_2$ nAChRs (Kendziorra et al., 2011).

Thus, there is considerable interest in the memory-enhancing and/or therapeutic potential in cognitive dysfunction of a highly potent and selective $\alpha_4\beta_2$ nAChR partial agonist like varenicline, which is approved by the FDA and in the European Union as a smoking cessation aid. Varenicline has approximately 20-fold higher affinity for human $\alpha_4\beta_2$ nAChRs than nicotine and binds with much lower affinity to other nicotinic and non-nicotinic central receptors (Rollema et al., 2007). Beside its partial agonist activity at $\alpha_4\beta_2$ nAChRs, varenicline is also considered a (much weaker) agonist at other heteromeric neuronal nicotinic receptors (e.g., α_3-and α_6–containing receptors) and the homomeric α_7 nAChR as well as a potent serotonin 5-HT3 receptor (Mihalak et al., 2006; Lummis et al., 2011).

Pro-cognitive effects of varenicline have in fact been found in animal models of various neuropsychiatric diseases. For example, varenicline enhanced hippocampal theta power and reversed drug-induced sensory gating deficits used in rodents to model cognitive dysfunction in schizophrenia (Rollema et al., 2009). The drug also improved performance in the sustained attention task under challenging distractor conditions similar to conditions in ADHD and in a novel object recognition (NOR) paradigm in rats following acute administration (Rollema et al., 2009). Moreover, in a clinical study, double-blind placebo-controlled adjunctive treatment with varenicline with atypical antipsychotic medication significantly improved neurocognitive functions, primarily of the frontal-executive type, in patients with schizophrenia (Shim et al., 2012). However, varenicline did not improve cognition in mild-to-moderate AD in a recent clinical trial (Kim et al., 2014).

The present study was conducted to investigate the effect of varenicline specifically on declarative memory processes in both cognitively normal mice and after scopolamine-induced amnesia. It was also analyzed whether varenicline altered the central $\alpha_4\beta_2$ nAChR protein and mRNA regulation. Sub-chronic varenicline has already been shown to further increase nicotine-induced $\alpha_4\beta_2$ nAChR upregulation in rat brain (Hussmann et al., 2014). Moreover, increased [^{3}H]epibatidine radioligand binding to nAChR protein following varenicline treatment of mice has been reported (Hussmann et al., 2012; Turner et al., 2011). However, to the knowledge of the authors, data on the direct effect of varenicline on the expression of the respective subunits are lacking.

2. Material and methods

2.1. Animals

All studies were performed in accordance with the guidelines of the German Animal Protection Law and were approved by local ethical control authorities. All efforts were made to minimize animal suffering, and to reduce the number of animals used. Male C57BL/6 mice (4–6 months old, numbers per group are given in Figure legends), bred in the University of Düsseldorf's Animal Facility, were used. Mice were housed in groups of 3–6 mice until 1 week prior to the beginning of the experiments. Thereafter, the mice were single-housed in standard translucent plastic cages (22 × 16 × 13 cm) with metal covers and dust-free sawdust bedding under standard laboratory conditions with a reversed 12 h light-dark cycle (lights on from 07:00 p.m. to 07:00 a.m.) with access to food and water *ad libitum*. Behavioral experiments were carried out during the animals' active period span, i.e., the artificial dark time span between 07:00 a.m. to 07:00 p.m.

2.2. Cell culture

Brain endothelial cells (b.END3) were purchased by ATCC (CRL-2299™, Wesel, Germany) and hippocampal HT-22 cells (prepared from C57BL/6 mice) were a kind gift from Heinz Mühlensiepen, Institute of Medicine, Research Center Jülich, Germany. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad CA) containing fetal calf serum (FBS, Gibco, Karlsruhe, Germany) and penicillin/streptomycin (PAA Laboratories, Pasching, Germany). All experiments with b.END3 were performed at passages 33–35, while HT-22 cells were used at passages 3–5. Incubation experiments with cell cultures were performed using sterile solutions of varenicline (10 nM–100 μM) in PBS (Gibco, Karlsruhe, Germany) for 24 h and 48 h.

2.3. Western blots

Expression of α_4 nAChR and β_2 nAChR protein subunits were determined in total lysates and membrane fractions of HT-22 cells and in hippocampus, neocortex and aortic mouse tissues prepared as described previously (Dao et al., 2011; Gordon, 1991). Cells and tissues were lysed in RIPA buffer (Roche, Mannheim, Germany). Western blotting was performed using commercially available polyclonal goat antibodies against α_4 nAChR and β_2 nAChR protein subunits (Abcam, Cambridge, UK, specifications are given in Supplementary Table 1) and antibodies against actin and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH, both from Sigma Aldrich, München, Germany; specifications are given Supplementary Table 2). The specificity of the antibodies has been tested using brain samples of α_4 (Marubio et al., 1999) and β_2 (Picciotto et al., 1995) knockout mice (Supplementary Fig. 1) which were kindly provided by Andrew Tapper (University of Massachusetts Medical School, Worcester, MA, USA) and Marina Picciotto (Yale University School of Medicine, New Haven, CT, USA), respectively. Blots were subsequently challenged either with a horseradish peroxidase-conjugated anti-goat antibody or anti–mouse antibody (LI-COR Biosciences, Bad Homburg, Germany), respectively. All blots were standardized to actin or GAPDH following a stripping procedure, i.e. figures of example blots were cut for proteins and loading controls. Comparative quantitative evaluation was performed with signals appearing on the same blot only.

2.4. Real-time PCR

Total RNA from cells and mouse tissues was isolated using the QIAshredder and RNeasy kit (Qiagen, Hilden, Germany), including an on-column DNAse digestion. Complementary DNA was synthesized from 1 μg total RNA by a commercially available kit (RevertAid™, H Minus First Strand cDNA Synthesis Kit, Life Technologies GmbH, Darmstadt, Germany) according to the manufacturer's protocol. Real time RT-PCR was performed with TaqMan® Gene Expression Assays (Applied Biosystems, Weiterstadt Germany). Specific intron spanning primers were selected using the software “Primer 3” (Koressaar and Remm, 2007) and were purchased (MWG, Ebersberg, Germany) for detection of α_4 nAChR mRNA (forward: 5′-CGG CCA GTA GCC AAT ATC TC, located in exon 2 and reverse 5′-CAG GTT GGT CGT CAT CAT CT, located in exon 3) and β_2 nAChR mRNA (forward: 5′-GGG AAG ATT ATC GCC TCA CA, located on exon 4 and reverse: 5′-TGC CGT CAG CAT TGT TGT AT, located on exon 4 and exon 5). For normalization purpose, co-amplification of

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