Neuropharmacology 64 (2013) 108–113

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Dopamine D_2-agonist Rotigotine effects on cortical excitability and central cholinergic transmission in Alzheimer's disease patients

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ARTICLE INFO

Article history:
Received 25 February 2012
Received in revised form
6 July 2012
Accepted 8 July 2012

Keywords:
Alzheimer's disease
Dopamine
D_2-agonists
SLAI
SICI
TMS

ABSTRACT

Dopamine is a neurotransmitter involved in several brain functions ranging from emotions control, movement organization to memory formation. It is also involved in the regulation of mechanisms of synaptic plasticity. However, its role in Alzheimer's disease (AD) pathogenesis is still puzzling. Several recent line of research instead indicates a clear role for dopamine in both amyloid β formation as well as in cognitive decline progression. In particular it has been shown that dopamine D_2-like receptors (namely D_3 and D_2) could be mostly responsible for dopamine dysfunction in AD. Here we aimed to study the effects of the dopamine agonist Rotigotine on cortical excitability and on central cholinergic transmission in cases of AD. Rotigotine is a dopamine agonist with a pharmacological profile with high affinity for D_3 and D_2 receptors. We used paired pulse protocols assessing short intracortical inhibition (SICI) and intracortical facilitation (ICF) to asses cortical excitability over the primary motor cortex and Short Latency Afferent Inhibition (SLAI) protocols, to verify the effects of the drug on central cholinergic transmission in a group of AD patients compared to age-matched controls. We observed that rotigotine induces unexpected changes in both cortical excitability (increased) and central cholinergic transmission (restored) of AD patients. These unexpected effects might depend on the dopamine D_2-like receptors dysfunction previously described in AD brains. The current findings could indicate that future strategies aimed to ameliorate symptoms of the related AD cognitive decline could also involve some dopaminergic drugs.

This article is part of a Special Issue entitled 'Cognitive Enhancers'.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia worldwide. It is characterized by the presence of extracellular deposits of amyloid protein, senile plaques, and intracellular fibrillary tangles. These features are responsible for progressive synaptic disarrangement, impairment of neurotransmission and cell loss. Several neurotransmitters are involved in neurodegenerative process, and their progressive deficit are responsible for symptoms of cognitive decline (see Francis, 2005; Martorana et al., 2010). Despite its major role in synaptic plasticity mechanisms, the involvement of dopamine (DA) in AD and in symptoms of cognitive decline is still puzzling. DA is synthesized in midbrain neurons and diffusely innervates hippocampus, neocortex, and basal ganglia. DA acts through five different types of receptors, traditionally distinct in two main subclasses: D_1-like (comprising the D_1 and the D_5 receptors) and D_2-like (comprising the D_2, D_3 and the D_4 receptors). Experimental evidences demonstrated that both at cortical and basal forebrain level DA is anatomically related to pyramidal neurons, GABA interneurons and also to cholinergic projections from the basal forebrain (Goldman-Rakic et al., 1999, 2000; Paspalas and Goldman-Rakic, 2005; Berlanga et al., 2005; Zhang et al., 2009). This network expresses both subclasses of DA receptors, and their activation is responsible for dopaminergic control of cortical activity. In particular, by acting through the D_2-like receptors, DA reduces cortical excitability (Gulledge and Jaffe, 1998; Tseng and O'Donnell, 2007; Kröner et al., 2007; Hosp et al., 2009; Molina-Luna et al., 2009), while via D_3 reduces cortical acetylcholine release (Millan et al., 2004, 2007; Lacroix et al., 2003). In AD the dysfunction of dopaminergic transmission has been hypothesized

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0028-3908/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuropharm.2012.07.015

as a new player in the patho-physiology of AD (Itoh et al., 1996; Kemppainen et al., 2003; Kumar and Patel, 2007; Martorana et al., 2010; Mura et al., 2010b). Localization studies of DA receptors showed preferential marked decrease of D_2-like receptors in the hippocampus and in the frontal cortex of AD brains (Kemppainen et al., 2003; Kumar and Patel, 2007). Moreover, recent transcranial magnetic stimulation (TMS) studies performed over the primary motor cortex showed with sufficient clarity that in AD patients DA dysfunction can be detected at early stages of the disease, even in absence of extrapyramidal signs. In particular, we recently found that in AD patients the altered motor cortical excitability supposed to be dependent on altered cortical GABAergic transmission (Di Lazzaro et al., 2002, 2004) is modulated by DA (Martorana et al., 2008). More interestingly, we also reported that the central cholinergic transmission, measured using the short latency afferent inhibition (SLAI) recordings (see Tokimura et al., 2000; Di Lazzaro et al., 2002), can be also restored by dopamine in AD patients (Martorana et al., 2009). However, which of the different DA receptors could be involved in these mechanisms remains still to be explored. Thus aim of this work was to study the effects of DAD_2-agonist on motor cortical excitability using standard paired pulse protocols assessing short intracortical inhibition (SICI) and intracortical facilitation (ICF) and on cholinergic transmission using SLAI paradigm, in a group of AD patients compared to age-matched healthy controls. In this work we choose the dopamine D_2 agonist Rotigotine (Neupro patch, UCB pharma) given its very high affinity for D_3 and D_2 (and low affinity for D_1 and D_5.). Due to this pharmacological selectivity like Rotigotine appears particularly suitable to study the effects of D_2-like receptor on human cortex activity. This would be particularly interesting since DAD_2 like receptors density progressively declines with ageing (Volkow et al., 1996; Ishibashi et al., 2009) and more severely in cases of AD (Joyce et al., 1998; Kemppainen et al., 2003).

2. Methods

2.1. Subjects

We examined 17 patients with a diagnosis of probable AD according to the NINCDS-ADRDA criteria (Varma et al., 1999) and 8 neurologically healthy age matched control subjects. The mean (SD) age of the patients was 69.4 (±4.8) years, while that of controls was 71.7 (±4.49) years. All patients underwent a complete clinical investigation, including medical history, neurological examination, mini mental state examination (MMSE), a complete blood screening (including routine exams, thyroid hormones, level of B_{12}), neuropsychological examination (see Pierantozzi et al., 2004) a complete neuropsychiatric evaluation and neuroimaging consisting of magnetic resonance imaging (1.5 T MRI). Exclusion criteria were the following: 1) patients with isolated deficits and/or unmodified MMSE (≥25/30) on revisit (6, 12, 18 months follow-up), patients with clinically manifest acute stroke in the last 6 months showing an Hachinsky scale > 4, and a radiological evidence of sub-cortical lesions. None of patients revealed pyramidal and/or extrapyramidal signs at the neurological examination. At the time of enrolment, in the 30 days before participating in this study, none of the patients had been treated with drugs that might have modulated cerebral cortex excitability such as antidepressants, or any other neuroactive drugs (i.e. benzodiazepines, anti-epileptic drugs or neuroleptics), and they had not been treated with cholinesterase inhibitors. The study was performed according to the Declaration of Helsinki and approved by the ethics committee of the Tor Vergata University in Rome. All AD patients showed a cognitive profile consistent with mild dementia, as assessed by a neuropsychological evaluation including the MMSE and a standardised neuropsychological battery (Carlesimo et al., 1996). On the MMSE, AD patients scored a mean of 22.8 (±4.3) and Clinical Dementia Rating (CDR) was 1.3 (±1.21). All participants or their legal guardian gave the written informed consent after receiving an extensive disclosure of study. The Local Ethic Committee approved the study procedures.

2.1.1. Drug administration

All participants were tested before and after Rotigotine administration. The pharmacological profile of Rotigotine shows a very high affinity for D_3 and D_2, and low affinity for D_1 and D_5. To guarantee the reliability of TMS results, physicians involved in recordings were completely blinded to the kind of experiments used in this protocol. None of the patients receiving Rotigotine complained for side effects. In experiment 1, performed in eleven AD patients and in eight age matched controls, Rotigotine was administered four days before the recordings at the dose of 4 mg patch (Neupro, UCB pharma).

In experiment 2, six AD patients were recorded four times once every week in the following conditions: at baseline, and four days after the administration of 2, 4 and 6 mg patch. The patch was applied on Monday and recordings were performed on Friday. In all participants Rotigotine peripheral effects were prevented with a 3-day Domperidone treatment (60 mg/day). Domperidone was administered two days before first Rotigotine administration and was stopped two days after last TMS recording.

2.1.2. TMS procedure

Single and paired TMS of the motor cortex of both hemispheres were performed with a 9 cm figure-of-eight coil connected with one or two Magstim 200 stimulators (The Magstim Company, Whitland, UK) via one Bistim module. The magnetic stimuli had a nearly monophasic pulse configuration, with a rise time of ~100 μs. The coil was placed at the optimal position for eliciting MEPs from the contralateral FDI muscle. The optimal position was marked on the scalp with a felt pen to ensure the identical placement of the coil throughout the experiment. The handle of the coil pointed backward and was perpendicular to the presumed direction of the central sulcus, about 45 deg to the midsagittal line. The direction of the induced current was from posterior to anterior, and was optimal to activate the motor cortex transsynaptically (Werhahn et al., 1994). The resting motor threshold (RMT) was defined as the lowest intensity that produced MEPs of >50 μV in at least five of 10 trials with the muscles relaxed (Rossini et al., 1994). The active motor threshold (AMT) was defined as the lowest intensity that produced MEPs of >200 μV in at least five of 10 trials when the subject made a 10% of maximum contraction using visual feedback (Rothwell, 1997). Determination of RMT and AMT was done in step width of 1% of MSO. SICI and ICF were tested using paired TMS with a subthreshold conditioning stimulus (CS) preceding a suprathreshold TS (Kujirai et al., 1993; Rothwell, 1997). Subthreshold CS stimulus was set at 80% AMT while the intensity of TS was adjusted to evoke a MEP of approximately 1 mV peak-to-peak in the relaxed left FDI. ISIs of 1, 2, 3, 5, 7, 10 and 15 ms were utilized to test SICI and ICF.

Short latency inhibition (SLAI) was studied using the technique that has been recently described (Sailer et al., 2003; Lang et al., 2008). Conditioning stimuli (CS) were single pulses (200 μs) of electrical stimulation applied through bipolar electrodes to the right median nerve at the wrist (cathode proximal). The intensity of the conditioning stimulus was set at just over motor threshold for evoking a visible twitch of the thenar muscles. The intensity of the test cortical magnetic stimulus was adjusted to evoke a muscle response in relaxed right FDI with an amplitude of approximately 1 mV peak to peak. The conditioning stimulus to the peripheral nerve preceded the magnetic test stimulus by different interstimulus intervals (ISI). Interstimulus intervals (ISIs) were determined relative to the latency of the N20 component of the somatosensory evoked potential induced by stimulation of the right median nerve. The active electrode for recording the N20 potential was attached 3 cm posterior to C3 (10–20 system) and the reference was 3 cm posterior to C4. Five hundred responses were averaged to identify the latency of the N20 peak. ISIs from the latency of the N20 plus 2 ms to the latency of the N20 plus 8 ms were investigated in steps of 2 ms. Ten stimuli were delivered at each ISI. The subject was given audiovisual feedback at high gain to assist in maintaining complete relaxation. The inter-trial interval was set at 5 s (±10%), for a total duration of approximately five minutes. Measurements were made on each individual trial. The mean peak-to peak amplitude of the conditioned MEP at each ISI was expressed as a percentage of the mean peak-to-peak amplitude size of the unconditioned test pulse in that block. To test if short latency inhibition was sensitive to changes because of Rotigotine activity we examined the motor threshold and the SLAI in patients at baseline and after four days of exposure to 4 mg of Rotigotine patch.

2.2. Data analysis

In experiment 1 for SICI and ICF we performed a repeated measures analysis ANOVA with GROUP (AD vs. healthy subjects) as between subjects factor and ISI (1, 2, 3, 5, 7, 10, 15 ms) and CONDITION (pre vs. post Rotigotine 4 mg) as within subjects factors. For SLAI the electrophysiological parameters of AD patients were compared with those of controls by means of repeated measures ANOVA with GROUP (AD vs. healthy subjects) as between subjects factor and ISI (−4, 0, +4 and +8 ms plus the latency of the N20) and CONDITION (pre vs. post Rotigotine 4 mg) as within subjects factors.

In experiment 2 for SICI and ICF we performed a repeated measures analysis ANOVA with ISI (1, 2, 3, 5, 7, 10, 15 ms) and CONDITION (pre vs. post Rotigotine 2 mg vs. post Rotigotine 4 mg vs. post Rotigotine 6 mg) as within subjects factors. For SLAI the electrophysiological parameters of AD patients were analysed by means of repeated measures ANOVA with ISI (−4, 0, +4 and +8 ms plus the latency of the N20) and CONDITION (pre vs. pre vs. post Rotigotine 2 mg vs. post Rotigotine 4 mg vs. post Rotigotine 6 mg) as within subjects factors.

When a significant main effect was reached, paired *t*-tests with Bonferroni correction were employed to characterize the different effects of the specific ISIs. For all statistical analyses, a p value of <0.05 was considered to be significant. Mauchly's test examined for sphericity. The Greenhouse–Geisser correction was used for non-spherical data.