The response of the autonomic nervous system to the cholinesterase inhibitor, donepezil

Hiroyuki Umegaki, Oyun Khookhor

Department of Geriatrics Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan

Correspondence to: Hiroyuki Umegaki, MD., PhD.
Department of Geriatrics Nagoya University Graduate School of Medicine
65 Tsuruma-cho, Showa-ku, Nagoya, Aichi, 466-8550, Japan.
TEL: +81-52-744-2364; FAX: +81-52-744-2371;
E-MAIL: umegaki@med.nagoya-u.ac.jp

Submitted: 2013-03-12 Accepted: 2013-05-15 Published online: 2013-08-03

Key words: **donepezil; acetylcholine; acethylcholinesterase; heart rate variability; autonomic nervous system**

Neuroendocrinol Lett 2013; **34**(5):383–387 **PMID:** 23922048 NEL340513A10

Abstract

OBJECTIVE: Donepezil, a cholinesterase inhibitor (ChEI) that is widely used to treat Alzheimer's disease, is thought to act by increasing extracellular acethylcholine (ACh) in the central nervous system. The effects of the administration of ChEIs on the autonomic nervous system, however, are complex and controversial.
METHODS: In this study we observed the effects of donepezil at a dose of 3 mg/kg intraperitoneally (i.p.) on the autonomic nervous system for two weeks using heart rate variability (HRV). In HRV, the high frequency (HF) band is influenced by cardiac parasympathetic tone, and the low frequency (LF) band is influenced by both sympathetic and parasympathetic activity. The ratio of LF and HF (LF/HF) is used for the assessment of the sympathetic nervous system.
RESULTS: In the current study the LF/HF was found to be significantly increased by day 1, but the magnitude of the response gradually decreased. The absolute power of HF increased after an i.p of donepezil of 3 mg daily in groups treated for 1 week and for 2 weeks compared to the single-dose groups.
CONCLUSION: Our results suggested that ChEI administration induced sympathetic nervous activation acutely, but chronic administration induced parasympathetic activation.

Abbreviations:

ACh	- acetylcholine
ChEI	- cholinesterase inhibitor
HRV	- heart rate variability
HF	- high frequency
LF	- low frequency
LF/HF	- low frequency to high frequency ratio

INTRODUCTION

Acetylcholine is one of the main neurotransmitters in the brain and is distributed in many brain regions including the hippocampus and cortex (Schliebs & Arendt 2006). A wide range of cognitive functions is modulated by the cholinergic system (Bentley *et al.* 2011), and the functions of memory and learning have been shown to be associated with the cholinergic system (Zeisel 1981). Alzheimer's disease (AD) is the most prevalent type of dementia world-wide. The degeneration in the central cholinergic system is supposed to be one of main underlying mechanisms of the cognitive dysfunction, including memory impairment, in this disease. Donepezil, which is a piperidine-derivative cholinesterase inhibitor (ChEI), has been prescribed to improve cognitive function in patients with AD (Dooley & Lamb 2000). The sys-

ORIGINAL ARTICLE

temic administration of donepezil increases the extracellular acetylcholine level of the hippocampus (Kosasa *et al.* 1999a) and the cortex (Kosasa *et al.* 1999b). The systemic administration of ChEIs like donepezil that penetrate into the central nervous system from the systemic blood flow is clinically used to improve the symptoms associated with AD.

We have reported that the central injection of neostigmine induced the elevation of plasma norepinephrine, presumably via spill-over from the sympathetic nervous terminals that reflected the sympathetic nervous activity (Umegaki *et al.* 2000; Umegaki *et al.* 2009; Khookhor & Umegaki 2013; Zhu *et al.* 2001a). Several other studies have also indicated that central activation of the cholinergic system induces sympathetic nervous activation (Risch *et al.* 1986) and blood pressure elevation (Savci *et al.* 1998; Milutinović *et al.* 2006). The intravenous injection of the short-acting ChEI activates the hypothalamo-pituitary-adrenal system through central cholinergic neurons in young humans (Rubin *et al.* 2006) and patients with Alzheimer's disease (Peskin *et al.* 1996). The central pathway, predominantly the central cholinergic system, probably leads to an increase in the activity of the sympatho-adrenal system (Savci *et al.* 1998). Donepezil, which elevates extracellular ACh in the brain, appears to activate the sympatho-adrenal system through the central nervous system mechanism (Sato *et al.* 2010).

On the other hand, all regions of the mammalian heart are innervated by parasympathetic (vagal) nerves, and the supraventricular tissues are more densely innervated than the ventricles. Vagal activation causes stimulation of cardiac muscarinic acetylcholine receptors that modulate pacemaker activity, and directly (in the atria) or indirectly (in the ventricles) the force of contraction (Dhein *et al.* 2001). Therefore, ChEIs may activate parasympathetic (vagal) nerves by the peripheral mechanism. The systemic administration of anti-AD ChEIs, which transition highly but not perfectly to the CNS, may activate the parasympathetic nervous system. Indeed, ChEIs-associated bradycardia has been reported (Hernandez *et al.* 2009).

Because most patients with AD are old and often have cardiovascular comorbidity, the effects on the cardiovascular system of centrally acting ChEI are very important.

Heart rate variability (HRV) is a useful non-invasive measure of autonomic activity. HRV is the temporal variation between sequences of consecutive heart beats. Power spectral analysis produces power bands that consist of high and low frequencies. The high frequency (HF) band is influenced by cardiac parasympathetic tone, the low frequency (LF) band is influenced by both sympathetic and parasympathetic activity, and the ratio of LF and HF (LF/HF) is used for the assessment of the sympathetic nervous system (Lombardi *et al.* 1996). Our previous study demonstrated that HRV could detect the changes in the autonomic nervous system induced by the central infection of ChEI (Khookhor & Umegaki 2013).

The goal of this study was to investigate the effects of the systemic administration of donepezil on the autonomic nervous system through HRV.

MATERIALS AND METHODS

Animals

Male Wistar/ST rats (Keary Co., Nagoya, Japan), 9 weeks of age (270–300 g), were housed at a room temperature of 23±1°C and relative humidity of 55±10%, under a 12-h light/dark cycle (lighting from 6:00 to 18:00) with standard diet pellets (Oriental Yeast Co., Ltd) and water available ad libitum. This study was approved by the Animal Care and Use Committee of Nagoya University.

Procedures

Thirty-four rats were divided into 2 groups (16 in the treatment group and 18 in the control group). Donepezil hydrochloride was provided from Eisai Co Ltd. (Tokyo, Japan). The treatment group was further divided into 3 subgroups, and donepezil 3 mg/kg/ml saline was intraperitoneally (i.p.)-administered as follows (Kosaka *et al.* 1999): (1) group - single dose (n=6), (2) group - daily for 7 days (n=5), and (3) group - daily for 14 days (n=5). In the control group (n=18) was only saline was i.p.-administered. All reagents were of biochemical grade.

Heart rate variability

HRV was measured at day 1, day 7, and day 14 after the administration of donepezil for 60 minutes. The AD Instruments HRV module signal was recorded using commercial software, with sampling at 50 Hz. A time spine of 40 minutes of RR interval data were digitized and stored on a computer for subsequent off-line analysis (Lab Chart® 7, AD Instruments, Pty Ltd., Australia).

The colleceted data were analyzed using customized Lab Chart software to obtain spectral components of HRV. Spectral components for HRV analysis were expressed as absolute units and normalized units and calculated as power in the LF (0.04–0.15 Hz), and the HF (0.15–0.40Hz) range was calculated for HRV.

Statistical analysis

The ratios of the HRV parameters at each time point after the injection of donepezil to those at the baseline (just before the injection, 0 minutes) were analyzed using a two-way repeated measures ANOVA followed by a post-hoc Fisher's PLSD test. For all inferential analyses, the probability error was set at 0.05. Results were reported as means ± standard deviation unless otherwise noted. Statistical analysis of variance was conducted to test for differences in frequency domain between the treatment groups and control group.

RESULTS

The HRV parameters at day 1, day 7, and day 14 are shown in Tables 1, 2, 3, respectively. The frequency domain parameters of heart rate variability (HRV) were significantly increased as determined by LF/HF on the first day, but the activation was attenuated at day 7 and day 14. The ratios of the HRV parameters after donepezil injection to those at baseline are shown in Figure 1 (LF/HF) and Figure 2 (HF) (LF/HF ratios for day 1 vs. day 14 and day 1 vs. day 7, p=0.0088 and p=0.0247, respectively). HF absolute power increased after the injection of donepezil in the groups treated with 3 mg daily for 1 week and for 2 weeks compared to the single dose group (HF ms^2 for day 1 vs. day 14 and day 14 vs. day 7, p=0.0097 and p=0.0049, respectively).

Tab. 1. HRV variablities after the *i. p.* injection of donepezil at the dose of 3mg/kg or saline at day 1.

	0min	10min	20min	30min	40min
LF/HF(ratio)					
Saline	2.188±0.31	2.652±0.52	3.576±0.46	2.703±0.58	2.622±0.99
Donepezil 3 mg/kg	1.842±1.23	3.965±2.76	6.666±2.53	4.048±0.54	2.899±0.49
Low frequency HRV (ms^2)					
Saline	0.040±0.02	0.056±0.02	0.077±0.06	0.070±0.06	0.110±0.13
Donepezil 3 mg/kg	0.033±0.02	0.099±0.10	0.207±0.12	0.110±0.07	0.096±0.63
High frequency HRV (ms^2)					
Saline	0.017±0.01	0.018±0.00	0.022±0.01	0.030±0.02	0.040±0.03
Donepezil 3 mg/kg	0.020±0.01	0.024±0.01	0.029±0.02	0.023±0.01	0.027±0.02

Tab. 2. HRV variablities after the *i. p.* injection of donepezil at the dose of 3 mg/kg or saline at day 7.

	0min	10min	20min	30min	40min
LF/HF(ratio)					
Saline	1.418±0.34	1.456±0.48	2.044±0.41	1.559±0.09	1.710±0.42
Donepezil 3 mg/kg	1.952±0.74	2.941±1.22	5.657±2.79	2.347±0.87	2.134±0.81
Low frequency HRV (ms^2)					
Saline	0.051±0.02	0.088±0.06	0.158±0.16	0.136±0.01	0.104±0.04
Donepezil 3 mg/kg	0.146±0.19	0.082±0.04	0.137±0.07	0.088±0.02	0.070±0.02
High frequency HRV (ms^2)					
Saline	0.033±0.01	0.060±0.03	0.046±0.03	0.088±0.00	0.058±0.03
Donepezil 3 mg/kg	0.033±0.01	0.033±0.01	0.033±0.02	0.050±0.03	0.033±0.01

Tab. 3. HRV variablities after the *i. p.* injection of donepezil at the dose of 3 mg/kg or saline at day 14.

	0min	10min	20min	30min	40min
LF/HF(ratio)					
Saline	2.649±0.84	2.482±0.90	3.026±1.36	2.860±1.10	2.289±0.53
Donepezil 3 mg/kg	2.049±1.39	2.400±1.70	4.112±2.94	2.263±1.32	1.575±1.04
Low frequency HRV (ms^2)					
Saline	0.054±0.02	0.085±0.03	0.083±0.02	0.076±0.05	0.073±0.03
Donepezil 3 mg/kg	0.089±0.11	0.100±0.09	0.156±0.12	0.114±0.08	0.095±0.08
High frequency HRV (ms^2)					
Saline	0.046±0.05	0.024±0.01	0.030±0.01	0.025±0.01	0.032±0.01
Donepezil 3 mg/kg	0.032±0.03	0.046±0.03	0.057±0.04	0.064±0.04	0.069±0.04

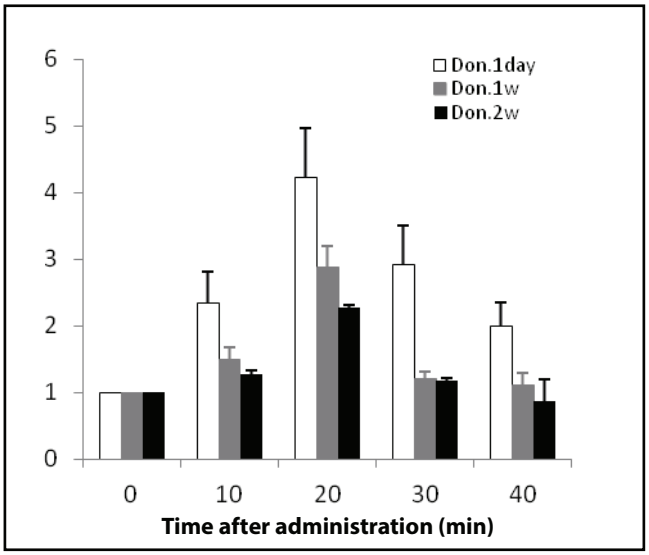

Fig. 1. Changes in time course of LF/HF ratio between day 1 vs. day14 and day1 vs. day 7 significantly different p=0.0088 and p=0.0247, respectively.

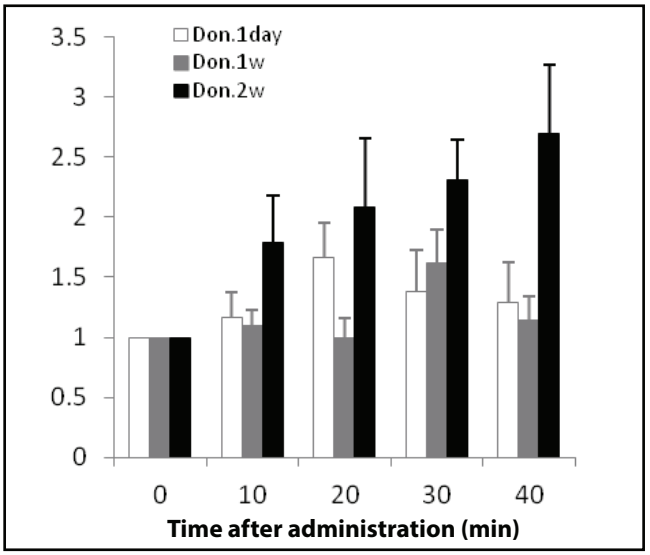

Fig. 2. Changes in time course of HF absolute power HF day1 vs. day 14 and day 14 vs. day 7 significantly different p=0.0097 and p=0.0049, respectively.

DISCUSSION

In the current study we demonstrated that systemic administration of donepezil, a ChEI, induced the sympathetic nervous activation measured by HRV, and chronic administration for 2 weeks reduced the sympathetic nervous activation but induced parasympathetic nervous activation.

McLaren *et al.* reported that the oral administration of donepezil induced the sympathetic nervous activation measured by HRV in dementia patients (McLaren *et al.* 2003). The current results partly agreed with this finding. The activation of the sympathetic nervous system may occur through the central mechanism. On the other hand, the administration of donepezil may result in peripheral cholinergic side-effects that include vagal activation (Nordberg & Svensson 1998) ; these side effects may result from peripheral direct cholinergic activation peripheral direct cholinergic activation.

The current study suggested that donepezil administration induced both the sympathetic nervous activation due to central cholinergic stimulation and the parasympathetic activation mediated by a peripheral mechanism, which showed different time-course profiles.

As we have previously reported, the central cholinergic stimulation elicited the activation of the HPA axis and the sympathetic nervous system (Umegaki *et al.* 2000; Khookhor & Umegaki 2013). In our previous study we reported that the intragastric administration of 3 mg/kg of donepezil significantly increased the plasma adrenocorticotropic hormone levels and c-Fos expression in the paraventricular nucleus (HPA axis activation) (Zhu *et al.* 2001a;b) and decreased the food intake on the first day (Umegaki *et al.* 2009). The increase in adrenocorticotropic hormone and the loss of appetite after intragastric administration of the drug were attenuated after daily administration for 2 weeks (Umegaki *et al.* 2009). The current observation of the attenuation of the sympathetic activity after the administration of donepezil is similar to the profile of the HPA axis activation. Pharmacologically, donepezil has a half-life of approximately 70 h, lending itself to once-daily administration (Wilkinson 1999); therefore, the cholinergic system in the CNS was chronically activated for the treatment period. This system may be desensitized by chronic cholinergic stimulation.

The time course of the parasympathetic nervous activation was different in two ways from that of the sympathetic nervous system; first, the peak of the activity was later, at 40 min after the administration, and second, it was reinforced from the first day to day 14. The mechanism of parasympathetic activation after the administration of donepezil remains to be elucidated The reasons for the reinforcement by means of chronic administration should also be investigated.

ChEIs reportedly induce atrial fibrillation (Constable *et al.* 1990; Bordier *et al.* 2005). Atrial fibrillation may be induced by the alteration of the activity of the autonomic nervous system (Park *et al.* 2012). Both the sympathetic and parasympathetic nervous systems can induce arrhythmia including atrial fibrillation. Age is the strongest risk factor for AD, and elderly patients often exhibit a comorbidity of heart disease; when prescribing ChEIs, medical care providers should keep in mind these effects on the autonomic nervous system. . In conclusion, we have demonstrated that acute administration of donepezil, a ChEI, induced sympathetic

nervous activation, and repeated administration of donepezil for 14 days attenuated the sympathetic activation and induced parasympathetic activation.

ACKNOWLEDGEMENT

Donepezil was generously provided by Eisai Co (Japan). The current study was supported by Grant-in Aid for Scientific Research form Japan Society for promotion of Science (C21591510).

REFERENCES

1 Bentley P, Driver J, Dolan RJ (2011). Cholinergic modulation of cognition: insights from human pharmacological functional neuroimaging. Prog Neurobiol. **94**: 360–368.
2 Bordier P, Lanusse S, Garrigue S, Reynard C, Robert F, Gencel L, et al (2005). Causes of syncope in patients with Alzheimer's disease treated with donepezil. Drugs Aging. **22**: 687–694.
3 Constable PD, Muir WW 3rd, Freeman L, Hoffsis GF, St Jean G, Welker FH (1990). Atrial fibrillation associated with neostigmine administration in three cows. J Am Vet Med Assoc. **196**: 329–332.
4 Dooley M, Lamb HM (2000). Donepezil: a review of its use in Alzheimer's disease. Drugs Aging. **16**: 199–226.
5 Dhein S, van Koppen CJ, Brodde OE (2001). Muscarinic receptors in the mammalian heart. Pharmacol Res. **44**: 161–182.
6 Hernandez RK, Farwell W, Cantor MD, Lawler EV (2009). Cholinesterase inhibitors and incidence of bradycardia in patients with dementia in the Veterans Affairs New England healthcare system. J Am Geriatr Soc. **57**: 1997–2003.
7 Khookhor O, Umegaki H (2013). The cholinergic stimulation of the hippocampus induced the activation of the sympathetic nervous system. Neuroendocrinol lett. **34**: 58–61.
8 Kosasa T, Kuriya Y, Matsui K, Yamanishi Y (1999a). Effect of donepezil hydrochloride (E2020) on basal concentration of extracellular acetylcholine in the hippocampus of rats. Eur J Pharmacol. **380**: 101–107.
9 Kosasa T, Kuriya Y, Yamanishi Y (1999b). Effect of donepezil hydrochloride (E2020) on extracellular acetylcholine concentration in the cerebral cortex of rats. Jpn J Pharmacol. **81**: 216–222.
10 Lombardi F, Malliani A, Pagani M, Cerutti S (1996). Heart rate variability and its sympatho-vagal modulation. Cardiovasc Res. **32**: 208–216.
11 McLaren AT, Allen J, Murray A, Ballard CG, Kenny RA (2003). Cardiovascular effects of donepezil in patients with dementia. Dement Geriatr Cogn Disord. **15**: 183–188.
12 Milutinović S, Murphy D, Japundzić-Zigon N (2006). Central cholinergic modulation of blood pressure short-term variability. Neuropharmacology. **50**: 874–883.
13 Nordberg A, Svensson AL (1998). Cholinesterase inhibitors in the treatment of Alzheimer's disease: a comparison of tolerability and pharmacology. Drug Saf. **19**: 465–480.
14 Risch SC, Janowsky DS, Mott MA, Gillin JC, Kalir HH, Huey LY, et al (1986). Central and peripheral cholinesterase inhibition: effects on anterior pituitary and sympathomimetic function. Psychoneuroendocrinology. **11**: 221–230.
15 Rubin RT, Rhodes ME, Miller TH, Jakab RL, Czambel RK (2006). Sequence of pituitary –adrenal cortical hormone responses to low-dose physostigmine administration in young adult women and man. Life Sci. **79**: 2260–2268.
16 Sato K, Urbano R, Yu C, Sato T, Jordan J, Robertson D, et al (2010). The effect of donepezil treatment on cardiovascular mortality. Clinical Pharmacol & Therapeutics. **88**: 335–338.
17 Savci V, Gurun MS, Cavin S, Ulus IH (1998). Cardiovascular effects of centrally injected tetahydroaminoacridine in conscious normotensive rats. Eur J Pharmacol. **346**: 35–41.
18 Schliebs R, Arendt T (2006). The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. J Neural Transm. **113**: 1625–1644.
19 Park HW, Shen MJ, Lin SF, Fishbein MC, Chen LS, Chen PS (2012). Neural mechanisms of atrial fibrillation. Curr Opin Cardiol. **27**: 24–28.
20 Peskin ER, Raskind MA, Wingerson D, Pascualy M, Thal LI, Dobie DJ, et al (1996). Hypothalamic-pituitary –adrenocortical axis responses to physostigmin: effects of Alzheimer's disease and gender. Biol Psychiatr. **40**: 61–68.
21 Umegaki H, Tamaya N, Shinkai T, Iguchi A (2000). The metabolism of plasma glucose and catecholamines in Alzheimer's disease. Exp Gerontol. **35**: 1373–1382.
22 Umegaki H, Yamamoto A, Suzuki Y, Iguchi A (2009). Responses of hypothalamo-pituitary-adrenal axis to a cholinesterase inhibitor. Neuroreport. **20**: 1366–1370.
23 Wilkinson DG (1999). The pharmacology of donepezil: a new treatment of Alzheimer's disease. Expert Opin Pharmacother. **1**: 121–135.
24 Zeisel S, Reinstein D, Corkin S, Growdon J, Wurtman R (1981). Cholinergic neurones and memory. Nature. **293**: 187–188.
25 Zhu W, Umegaki H, Yoshimura J, Tamaya N, Suzuki Y, Miura H, et al (2001a). The elevation of plasma adrenocorticotrophic hormone and expression of c-Fos in hypothalamic paraventricular nucleus by microinjection of neostigmine into the hippocampus in rats: comparison with acute stress responses. Brain Res. **892**: 391–395.
26 Zhu W, Umegaki H, Suzuki Y, Miura H, Iguchi A(2001b). Involvement of the bed nucleus of the stria terminalis in hippocampal cholinergic system-mediated activation of the hypothalamo--pituitary--adrenocortical axis in rats. Brain Res. **916:** 101–106.