ORIGINAL ARTICLE

The cholinergic stimulation of the hippocampus induced the activation of the sympathetic nervous system

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Abstract

OBJECTIVES: We previously demonstrated that the direct microinjection of cholinesterase inhibitor (neostigmine) into the hippocampus in rats activated the hypothalamo-pituitary –adrenal axis and increased the level of norepinephrine in the plasma. In the current study we tried to measure the effects of neostigmine injection into the hippocampus using the non-invasive measure of heart rate variability (HRV).

MATERIALS AND METHODS: After the hippocampal injection of neostigmine (50 nM and 125 nM) or saline as a control in rats, HRV measurement was performed for 30 min.

RESULTS: After the injection of neostigmine into the hippocampus, LF/HF, the index of the sympathetic nervous system, significantly increased.

CONCLUSIONS: The results of the current study indicated that stimulation of the hippocampal cholinergic system induced the activation of the sympathetic nervous system.

Abbreviations:

HPA	- Hypothalamo-pituitary-adrenal axis
ChEI	- cholinesterase inhibitor
CRH	- corticotropin-releasing hormone
PVN	- paraventricular nucleus
NE	- norepinephrine
HRV	- heart rate variability
LF/HF	- low frequency to high frequency
LF	- low frequency
SNS	- sympathetic nervous system
CNS	- central nervous system

INTRODUCTION

The central nervous system (CNS) controls the activity of the autonomic nervous system and the endocrine system to maintain homeostasis (Smith & DeVIto 1984; Loewy & Mackellar 1980). Many brain regions are involved in the control system (Loewy & Michael 1990). The hypothalamus is a center of neuroendocrine regulation and is also reciprocally interconnected with the autonomic system (Palkovits 1999). The paraventricular nucleus (PVN) of the hypothalamus is closely associated with stress responses (Herman *et al.* 2003) and controls of the autonomic nervous system (Smith & DeVito 1984). We have reported that the injection of neostigmine, a cholinesterase inhibitor (ChEI), into the hippocampus, which activates the cholinergic system via the increase of acetylcholine in the synaptic cleft, leads to the activation of the neurons in the PVN and also elevates both the plasma catecholamines including norepinephrine (NE), which reflects the activity of the sympathetic nervous system (SNS), and ACTH, which indicates the activation of the hypothalamo-pituitary-adrenocortical axis (HPA) via the bed nucleus stria terminalis (BNST) (Zhu *et al.* 2001a, b; Umegaki *et al.* 2000). Several other studies also indicate that central activation of the cholinergic system induces sympathetic nervous activation (Risch *et al.* 1986; MIlutinovic *et al.* 2006).

Heart rate variability (HRV) is a non-invasive measure of autonomic activity. HRV is the temporal variation between sequences of consecutive heart beats, and power spectral analysis produces power bands that consist of high and low frequencies. The high frequency band is influenced by cardiac parasympathetic tone, the low frequency (LF) band is influenced by both sympathetic and parasympathetic activity, and the ratio of low frequency to high frequency (LF/HF) is used for the assessment of the SNS (Lombardi *et al.* 1996).

As mentioned above, we have reported that hippocampal injection of ChEI induced NE elevation in plasma, and it is speculated that it is the spill-over of the sympathetic nervous terminal that suggests the activation of the sympathetic nervous activity. However, no studies have investigated whether the central pathway of cholinergic effects on autonomic nervous system can be detected by the measurement of HRV.

The aim of the current study was to detect the changes in the autonomic nervous system induced by central cholinergic stimulation through HRV measurement.

MATERIALS AND METHODS

Animals

Male Wistar/ST rats (Japan SLC, Hamamatsu, Japan), 9 weeks of age, (270–300 g), were housed at a room temperature of 23±1 °C and a 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

After a one-week acclimatization period, the rats underwent surgery 1 week before the experiments to implant a guide cannula (BAS, Tokyo, Japan) into the left dorsal hippocampus at the following coordinates: AP-0.8 mm, ML±1.5 mm, D-3.5 mm from Bregma in accordance with the Paxinos and Watson atlas (Paxinos & Watson 1986). Saline containing neostigmine methylsulfate (Sigma, neostigmine $5x10^{-8}$ mol/µl in saline, and $1.25x10^{-7}$ mol/µl in saline) or saline was microinjected in a volume of 1 ìl for 1 min by a CMA/100 Microinjection Pump (BSA, Tokyo, Japan) through the guide cannula into the left dorsal hippocampus. All reagents were of biochemical grade.

Heart rate variability

The HRV module (AD Instruments, Pty Ltd., Australia) signal was recorded using commercial software, sampling at 50 Hz. RR interval data over a time span of 30 minutes were digitized and stored on a computer for subsequent offline analysis (Lab Chart@ 7, AD Instruments, Pty Ltd., Australia).

The RR interval data were analyzed using customized Lab Chart software to obtain the spectral components of HRV. Spectral components for HRV analysis were expressed as absolute units and normalized units, calculated as Power in the LF (0.04–0.15 Hz), and the HF (0.15–0.40 Hz) range was calculated for HRV (Task Force of the European Society of Cardiology and the North America Society of Pacing and Electrophysiology.1996). The LF band reflects both sympathetic and parasympathetic activity. The HF band reflects cardiac vagal tonus and is associated with respiration-related heart rate changes (respiratory sinus arrhythmia). The LF/HF ratio reflects the sympathetic/parasympathetic activity ratio. The parameters of HRV were measured retrospectively.

Statistical analysis

Data were analyzed using a two-way repeated measures analysis of variance (ANOVA). For all 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 differences in the frequency domain between treatment groups and control groups.

RESULTS

The effects of the hippocampal injection of neostigmine on HRV were investigated in subgroups of rats (n=9 for 5×10^{-8} mol/µl, n=6 for 1.25×10^{-7} mol/µl of neostigmine) which were treated with microinjection into the hippocampus; another subgroup of saline-treated rats

(n=6) served as controls. The measurements of LF, HF, and LF/HF at baseline and after the hippocampal injection of neostigmine or saline are shown in Tables 1, 2 and 3, respectively. LF/HF increased after the injection with a peak response at 10 min after the injection. The profiles of LF/HF changes given as ratios to the baseline values are shown in Figure 1. Two-way repeated ANOVA revealed the main effects of time ($F=34.778$, $p<0.001$). The interaction of time and neostigmine dose was also statistically significant ($F=8.196$, $p<0.001$).

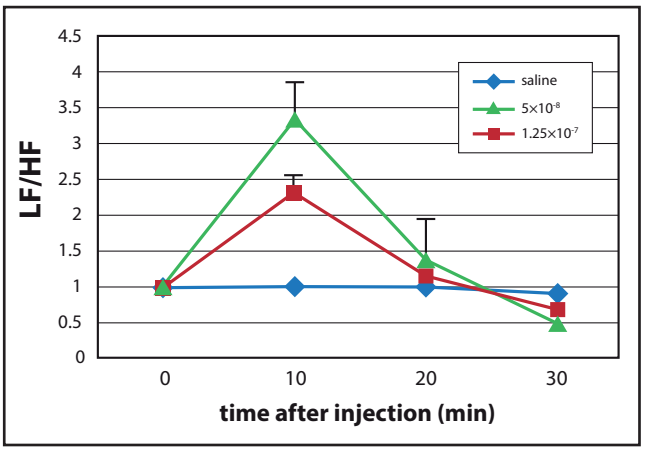

Fig. 1. Changes of LF/HF after the hippocampal injection of neostigmine (0, 5×10^{-8} mol/μl, 1.25×10^{-7} mol/μl).

DISCUSSION

In the current study we demonstrated that non-invasive measurement by HRV could detect the response of the SNS to the hippocampal activation of the cholinergic system.

Our previous reports showed that central injection of neostigmine induced the elevation of NE, presumably as a spillover from sympathetic nervous terminals (Zhu *et al.* 2001a, b; Umegaki *et al.* 2000). The responses associated with epinephrine and ACTH elevation in plasma resemble stress responses (Umegaki *et al.* 2000). The current study confirmed that stimulation of the cholinergic system in the hippocampus induces the activation of the autonomic system and the SNS.

HRV is a noninvasive method involving electrocardiography, and the present study showed that it could detect the responses of the autonomic nervous system that were induced by direct CNS stimulation. Considerable fluctuations of the autonomic nervous system are indeed induced by the control of CNS without peripheral stimulation. Thus HRV may have the potential to reflect the activities of some areas in the CNS. Indeed, sympathetic nervous activity could be a marker for the cholinergic activity in the hippocampus.

LF/HF is generally accepted as an index of cardiac sympathovagal balance (Task Force 1996). However, new researches suggested that LF power could reflect

Tab. 1. LF/HF after the hippocampal injection of neostigmine.

	baseline	10 min after injection	20 min after injection	30 min after injection
saline (ms^2)	1.98±0.72	1.97±0.66	1.93±0.59	1.77±0.58
neostigmine (50nM) (ms^2)	1.51±0.72	3.14±0.75	1.77±1.052	1.05±0.78
neostigmine (125nM) (ms^2)	1.84±1.37	5.49±3.35	1.96±1.61	0.78±0.87

Means±SD are shown.

Tab. 2. LF after the hippocampal injection of neostigmine.

	baseline	10 min after injection	20 min after injection	30 min after injection
saline (ms^2)	0.11±0.08	0.12±0.09	0.14±0.14	0.14±0.09
neostigmine (50nM) (ms^2)	0.22±0.35	0.16±0.06	0.24±0.29	1.88±4.23
neostigmine (125nM) (ms^2)	0.19±0.24	0.47±0.50	0.37±0.53	0.29±0.36

Means±SD are shown.

Tab. 3. HF after the hippocampal injection of neostigmine.

	baseline	10 min after injection	20 min after injection	30 min after injection
saline (ms^2)	0.16±0.30	0.06±0.04	0.06±0.06	0.07±0.04
neostigmine (50nM) (ms^2)	0.24±0.57	0.06±0.04	0.12±0.12	1.90±3.54
neostigmine (125nM) (ms^2)	0.21±0.42	0.05±0.04	0.25±0.30	0.40±0.48

Means±SD are shown.

baroreflex function, not exactly the only sympathetic innervation (Moak *et al.* 2009). Hence, the results in the current study should be reconfirmed by other methodology than HRV such as measurement of cardiac norepinephrine spillover.

The cholinergic system in the CNS undergoes major damage in Alzheimer's disease, and this system is closely associated with memory impairment in Alzheimer's. The ChEIs that inhibit cholinesterase in the brain with high selectivity are prescribed to treat Alzheimer's disease. The administration of these medicines may activate the SNS through the activation of the central cholinergic system according to the results of the current study. On the other hand, the systemic administration of ChEIs affects the parasympathetic nerve in the heart directly through the muscarinic receptors. A risk of bradycardia with the prescription of ChEIs for the treatment of Alzheimer's disease was reported in a large cohort study (McLaren *et al.* 2003). On the other hand, Mclaren et al reported that a ChEI used for Alzheimer's disease treatment, donepezil, induced sympathetic nervous activation, which agrees with the results of the current study (Wilkinson *et al.* 2004). These findings may be taken into consideration when ChEIs are administered in the elderly with cardiovascular comorbidities. ChEIs affect the parasympathetic nerve in the heart directly through the muscarinic receptors, and hippocampal cholinergic system activation may affect the SNS. The administration of ChEI may have complex effects on the autonomic nervous system.

In conclusion, the current study shows that the stimulation of the hippocampal cholinergic system induced the activation of the SNS and that the noninvasive method of HRV has the potential to detect these responses.

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Conflicts of Interest

There are no conflicts of interest.

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