33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Experimental 'Jet Lag' Causes Sympathoexcitation via Oxidative Stress through AT_1 Receptor in the Brainstem

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***Abstract*— Circadian disruptions through frequent transmeridian travel, rotating shift work, and poor sleep hygiene are associated with an array of physical and mental health maladies, including the abnormal autonomic nervous system. We have demonstrated that the oxidative stress through AT_1 receptor in the brain activates sympathetic nervous system. The aim of the present study was to determine whether experimental 'jet lag' causes sympathoexcitation via oxidative stress through AT_1 receptor in the cardiovascular center of the brainstem (rostral ventrolateral medulla; RVLM) or not. Experimental 'jet lag' was made to normotensive (Wister-Kyoto rat; WKY rat) and hypertensive rats (stroke-prone spontaneously hypertensive rats; SHRSP) by the exposure to a 12 hour phase advance for 5 days. In WKY, 'jet lag' increases blood pressure and the activity of sympathetic nervous system via oxidative stress through angiotensin II type 1 receptor in the RVLM for 2 days only, and the changes are improved at 3 day after the initiation of 'jet lag'. In SHRSP, 'jet lag' also increases blood pressure and the activity of sympathetic nervous system via oxidative stress through angiotensin II type 1 receptor in the RVLM, and the changes are greater compared to those in WKY, and are maintained for the period of 'jet lag'. These results suggest that experimental 'jet lag' causes sympathoexcitation via oxidative stress through AT_1 receptor in the brain, especially in hypertension.**

INTRODUCTION

Frequent transmeridian travel is known to cause an disturbance in circadian timing system [1, 2]. This disturbance is associated with a number of clinical pathologies, including a higher incidence of hypertension and cardiovascular disease [3, 4]. In mammals, the master circadian pacemaker is located in the suprachiasmatic nucleus (SCN) in the anterior hypothalamus, and the SCN generates endogenous oscillations with a period of approximately 24 hours [5]. At the cellular level, circadian rhythms are generated by 24-hour autoregulatory transcriptional feedback loops consisting of 'clock' genes and their protein products [6]. A recent study suggests that the circadian disruption lead to marked suppression of hippocampal cell proliferation and neurogenesis, associated with notable deficits in learning and memory [7].These results indicate that 'jet lag' causes the changes in neural structures and functions in the brain. In terms of the regulation of blood pressure and heart rate via sympathetic nervous system, central nervous system involved in baroreflex circuit is important [8]. However, it has not been determined whether the abnormalities in blood pressure and heart rate in 'jet lag' are due to the changes in central nervous system or not.

We have demonstrated that nitric oxide and oxidative stress in the brainstem regulates the activity of the sympathetic nervous system [9, 10]. Especially, in the brainstem, oxidative stress through the angiotensin II type 1 receptor in the rostral ventrolateral medulla (RVLM) causes the sympatho-excitation [11]-[13]. Taken together, we hypothesize that 'jet lag' might cause hypertension through the sympathoexcitation due to the oxidative stress in the RVLM. However, the mechanisms in which 'jet lag' causes hypertension or sympathoexcitation have not been fully determined.

The aims of the present study was to determine whether the experimental 'jet lag' causes sympathoexcitation or not, and if so, whether the experimental 'jet lag'-induced sympathoexcitation is due to the oxidative stress through AT_1 receptor in the RVLM or not. To do these aims, we made the experimental 'jet lag' model rats by the exposure to a 12 hour phase advance for 5 days.

PROCEDURES

Ethics statement

This study was reviewed and approved by the committee on ethics of Animal Experiments, Kyushu University Graduate School of Medical Sciences, and conducted according to the Guidelines for Animal Experiments of Kyushu University.

Animals

Adult male stroke-prone spontaneously hypertensive rats (SHRSP) and Wister-kyoto (WKY) rats maintained on a 14:10 light:dark (LD) cycle (Lights on at 0700 h) prior to the onset of the experiments, with a light

Manuscript submitted April 15, 2011, revised manuscript submitted June 20, 2011.

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intensity ranging from 100-300 lux at the level of each cage. All animals were maintained in a colony room and provided with ad libitum access to water and food.

Experimental 'Jet Lag'

WKY and SHRSP were divided into two groups, 'jet lag'-WKY, control-WKY, 'jet lag'-SHRSP, and control-SHRSP. 'Jet lag'-WKY and –SHRSP groups were exposed to a 12 hour phase advance for 5 days, while control-WKY and –SHRSP groups were remained in a 14:10 LD (lights on at 0700 hr) cycle for the same duration.

Measurement of Blood Pressure and Heart rate

The UA-10 telemetry system (Data Sciences International) was used to measure mean arterial pressure and heart rate. The surgical procedure has been described previously [9, 11]. Mean arterial pressure and heart rate were recorded continuously for 10 minutes every day in light and dark phase by a multichannel amplifier and signal converter.

Urinary Norepinephrine Excretion As an Parameter of the Activity of Sympathetic Nervous System

As the parameter of the activity of the sympathetic nervous, we measured the urinary norepinephrine concentration by high-performance liquid chromatography (HPLC), and calculated the urinary norepinephrine excretion for 24 hours [9]-[13].

Oxidative Stress in the RVLM

As an indicator of the oxidative stress in the RVLM, we measured thiobarbituric acid-reactive substances (TBARS) levels in the tissues obtained from the RVLM of each group at the end of the study as described in previous studies [11]-[13]. Moreover, to determine the TBARS levels in the RVLM at 2 day after the initiation of 'jet lag', we made the other 4 groups, 'jet lag' for 2 days-WKY, control-WKY, 'jet lag' for 2 days-SHRSP, and control-SHRSP.

Microinjection of Angiotensin II Type 1 Receptor Blocker into the RVLM

To inhibit the angiotensin II type 1 receptor in the RVLM locally, we microinjected losartan (1nmol), angiotensin II type 1 receptor blocker, into the bilateral RVLM of each group at the end of the study. Moreover, to determine the activity of the angiotensin II type 1 receptor in the RVLM at 2 day after the initiation of 'jet lag', we made the other 4 groups, 'jet lag' for 2 days-WKY, control-WKY, 'jet lag' for 2 days-SHRSP, and control-SHRSP. Each rat was anesthetized with sodium pentobarbital. A catheter was inserted into the femoral artery to record arterial blood pressure. A tracheal cannula was connected to a ventilator, and the rats were artificially ventilated. The rats were placed in a stereotaxic frame. The identification of the RVLM and the procedures of the microinjection were confirmed as described previously [11]-[13].

RESULTS

Blood Pressure and Heart rate

Fig.1 shows the results of mean arterial pressure. Prior to the experiments, mean arterial pressure and heart rate were significantly higher in SHRSP than in WKY both at light and dark phase (Fig. 1 and 2). For the rats, light phase is a rest phase, and dark phase is an active phase. In dark and light phase, mean arterial pressure and heart rate were significantly higher in 'jet lag'-WKY than in control-WKY at 1-2 day after the initiation of 'jet lag', and was similar in 'jet lag'-WKY and control-WKY at 3-5 day after the initiation of 'jet lag' (Fig. 1 and 2). In dark phase, mean arterial pressure and heart rate were similar in 'jet lag'-SHRSP and control-SHRSP (Fig. 1 and 2). However, in light phase, mean arterial pressure and heart rate were significantly higher in 'jet-lag'-SHRSP than in control-SHRSP for the 'jet lag' period (Fig. 1 and 2).

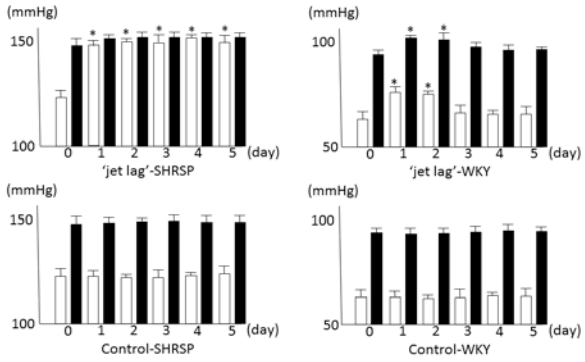

Fig 1. The results of mean arterial pressure in each group. White column indicates light phase, and black column indicates dark phase. N=5 for each. *P<0.05 vs control.

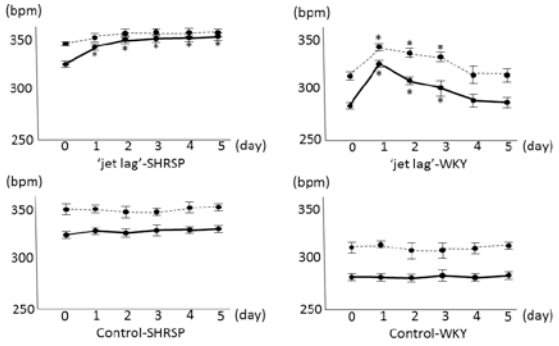

Fig 2. The results of the averages of heart rate in each group. Solid line indicates light phase, and dot line indicates dark phase. N=5 for each. *P<0.05 vs control.

Urinary Norepinephrine Excretion

Prior to the experiments, urinary norepinephrine excretion was significantly higher in SHRSP than in WKY (Fig. 3). In WKY, urinary norepinephrine excretion was significantly higher in 'jet lag'-WKY than in control-WKY at 1-2 day after the initiation of 'jet lag', and was similar in 'jet lag'-WKY and control-WKY at 3-5 day after the initiation of 'jet lag' (Fig. 3). In SHRSP, urinary Units norepinephrine excretion was significantly higher in 'jet lag'-SHRSP than in control-WKY for the period of 'jet lag' (Fig. 3).

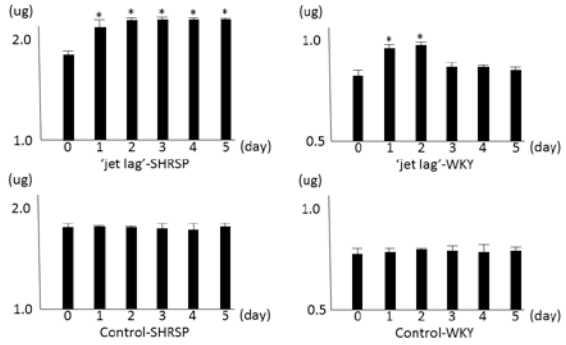

Fig 3. The results of the urinary norepinephrine excretion in each group for 5 days of jet lag. N=5 for each. *P<0.05 vs control.

Oxidative Stress in the RVLM

In WKY, TBARS in the RVLM was significantly higher in 'jet lag'-WKY than in control-WKY at 2 day after the initiation of 'jet lag', and was similar in 'jet lag'-WKY and control-WKY at 5 day after the initiation of 'jet lag' (Fig. 4). In SHRSP, TBARS in the RVLM was significantly higher in 'jet lag'-SHRSP than in control-WKY both at 2 and 5 day after the initiation of 'jet lag' (Fig. 4).

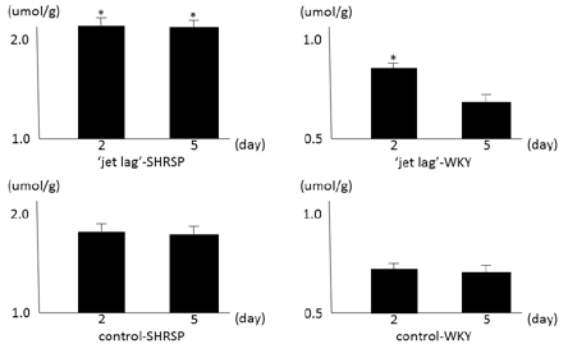

Fig 4. The results of the levels of TBARS in the RVLM of each group. N=5 for each. *P<0.05 vs control.

Microinjection of Angiotensin II Type 1 Receptor Blocker into the RVLM

In WKY, the depressor effect due to the microinjection of losartan into the RVLM was significantly greater in 'jet lag'-WKY than in control-WKY at 2 day after the initiation of 'jet lag', and was similar in 'jet lag'-WKY and control-WKY at 5 day after the initiation of 'jet lag' (Fig. 5). In SHRSP, the depressor effect was significantly greater in 'jet lag'-SHRSP than in control-WKY both at 2 and 5 day after the initiation of 'jet lag' (Fig. 5).

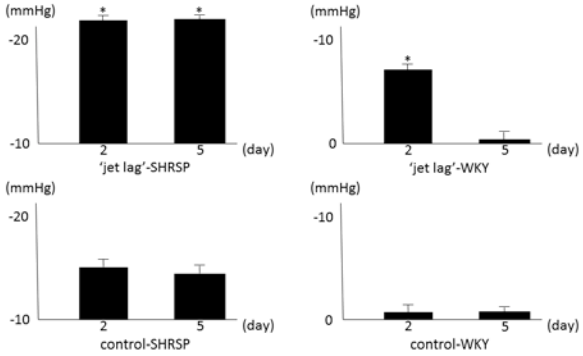

Fig 5. The results of the degree of the depressor effects due to the microinjection of losartan into the RVLM. N=5 for each. *P<0.05 vs control.

DISCUSSION

In the present study, we demonstrated that 1) in WKY, 'jet lag' increases blood pressure and the activity of sympathetic nervous system via oxidative stress through angiotensin II type 1 receptor in the RVLM for 2 days only, and the changes are improved at 3 day after the initiation of 'jet lag', 2) in SHRSP, 'jet lag' also increases blood pressure and the activity of sympathetic nervous system via oxidative stress through angiotensin II type 1 receptor in the RVLM, and the changes are maintained for the period of 'jet lag'. From these results, we consider that 'jet lag' causes sympathoexcitation via oxidative stress through angiotensin II type 1 receptor in the RVLM, and the 'jet lag'-induced sympathoexcitation is maintained and excessive in SHRSP. The clinical implications from the present study are that hypertension is a risk of 'jet lag'-induced sympathoexcitation, and that angiotensin II type 1 receptor blocker might be an effective agent of the treatment for 'jet lag'-induced sympathoexcitation.

The most important finding in the present study is that 'jet lag' activates the angiotensin II type 1 receptor in the brain. The activity of the sympathetic nervous system is regulated mainly by the angiotensin II type 1 receptor-induced oxidative stress in the RVLM [11]-[13]. Previous studies have suggested that 'jet lag' increases blood pressure [3]-[4]. Taken together, we consider that the mechanisms of 'jet lag'-induced hypertension and sympathetic activation might be due to the activation of the angiotensin II type 1 receptor in the brain. Furthermore, while the sympathoexcitation is tentative in WKY, the sympathoexcitation is maintained in SHRSP. We should consider that hypertension is a worsening factor of 'jet lag'-induced sympathoexcitation.

In terms of the treatment for 'jet lag'-induced sympathoexcitation, the target of the treatment might be angiotensin II type 1 receptor in the RVLM. To inhibit the angiotensin II type 1 receptor in the brain, in the present study, we performed the microinjection of the angiotensin II type 1 receptor blocker directly into the RVLM, and in our previous study, we performed the intracerebroventricular infusion of the angiotensin II type 1 receptor blocker [13]. In clinical aspects, oral administration of angiotensin II type 1 receptor blocker might be a novel agent, because some oral intake of angiotensin II type 1 receptor blocker affects the RVLM through the blood-brain barrier [10]. Moreover, we have also demonstrated that some other oral agents, especially statin, have the potential to inhibit the oxidative stress in the brain [10, 14]. In the further study, we should examine the effects of the oral administration of the angiotensin II type 1 receptor blocker s and / or statins on the 'jet lag'-induced sympathoexcitation.

The mechanisms in which 'jet lag' activates the angiotensin II type 1 receptor in the brain have not been determined in the present study. In hypertension, previous studies have suggested that the angiotensin II type 1 receptor in the brain is activated by the circulating angiotensin II and / or baroreflex circuit [8, 10]. In the present study, we did not determine the changes in the concentration of plasma angiotensin II and the baroreflex sensitivity. Further studies must be done to determine mechanisms in which 'jet lag' activates the angiotensin II type 1 receptor in the brain.

There are some limitations in the present study. First, we only examined the oxidative stress in the RVLM. The increase in oxidative stress in the brain of 'jet lag' may not be the unique phenomenon in the RVLM. However, in the regulation of sympathetic nerve activity, RVLM is the most important cite. Furthermore, in the RVLM, oxidative stress is the most powerful and important sympatho-exciting factor [11, 13]. From these reasons, we focused on the oxidative stress in the RVLM. Second, we did not perform the long-term RVLM-specific inhibition of AT_1 receptor. We must do the RVLM-specific knock down of AT_1 receptor in the future study.

CONCLUSION

The results from the present study suggest that experimental 'jet lag' causes sympathoexcitation via oxidative stress through AT_1 receptor in the brain, especially in hypertensive states.

APPENDIX

None.

ACKNOWLEDGEMENT

This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (B193290231).

REFERENCES

[1] A.J. Davidson, O. C. Castanon, T. L. Leise, P. C. Molyneux, M. E. Harrington, "Visualizing jet lag in the mouse suprachiasmatic nucleus and peripheral circadian timing system", Eur J Neurosci, vol. 29, pp. 171-180, 2009.

[2] E. S. Maywood, J. O'Neill, G. K. Wong, A. B. Reddy, M. H. Hastings, "Circadian timing in health and disease", Prog Brain Res, vol. 153, pp. 253-269, 2006.

[3] M. Ha, J. Park, "Shiftwork and metabolic risk factors of cardiovascular disease", J Occup Health, vol. 47, pp. 89-95, 2005.

[4] M. Kivimaki, M. Virtanen, M. Elovainio, A. Vaananen, L. K. Jarvinen L, "Prevalent cardiovascular disease, risk factors and selection out of shift work", Scand J Work Environ Health, vol. 32, pp. 204-208, 2006.

[5] R. Y. Moore, V. B. Eichker, "Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat", Brain Res, vol. 42, pp. 201-206, 1972.

[6] R. Chen, A. Schirmer, Y. Lee, H. Lee, V. Kumar, "Rhythmic PER abundance defines a critical nodal point for negative feedback within the circadian clock mechanisms", Mol Cell, vol. 36, pp. 417-430, 2009.

[7] E. M. Gibson, C Wang, S Tjho, N Khattar, L.J. Kriegsfeld, "Experimental 'jet lag' inhibits adult neurogenesis and produces long-term cognitive deficits in female hamsters", PloS ONE, vol. 5, e15267, 2010.

[8] P. G. Guyenet PG, "The sympathetic control of blood pressure", Nat Rev Neurosci, vol. 7. pp. 335-346, 2006.

[9] Y. Hirooka, K. Sakai, T. Kishi, A. Takeshita, "Adenovirus-mediated gene transfer into the NTS in conscious rats: A new approach to examining the central control of cardiovascular regulation", Ann N Y Acad Sci, vol. 940, pp. 197-205, 2001.

[10] Y. Hirooka, T. Kishi, K. Sakai, A. Takeshita, K. Sunagawa, "Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension", Am J Physiol, vol. 300, pp. R818-R826, 2011.

[11] T. Kishi, Y. Hirooka, Y. Kimura, K. Ito, H. Shimokawa, A. Takeshita, "Increased reactive oxygen species in rostral ventrolateral medulla contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats", Circulation, vol. 109, pp. 2357-2362, 2004.

[12] M. Nozoe, Y. Hirooka, Y. Koga, S. Araki, S. Konno, T. Kishi, T. Ide, K. Sunagawa K, "Mitochondria-derived reactive oxygen species mediate sympathoexcitation induced by angiotensin II in the rostral ventrolateral medulla", J Hypertens, vol. 26, pp. 2176-2184, 2008.

[13] T. Kishi, Y. Hirooka, S. Konno, K. Ogawa, K. Sunagawa, "Angiotensin II type 1 receptor-activated caspase-3 through ras/mitgen-activated protein kinase-extracellular signal-regulated kinase in the rostral ventrolateral medulla is involved in sympathoexcitation in stroke-prone spontaneously hypertensive rats", Hypertension, vol. 55, pp. 291-294, 2010.

[14] T. Kishi, Y. Hirooka, H. Shimokawa, A. Takeshita, K. Sunagawa, "Atorvastatin reduces oxidative stress in the rostral ventrolateral medulla of stroke-prone spontaneously hypertensive rats", Clin Exp Hypertens, vol. 30, pp. 1-9, 2008.