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Up-regulation of Na^+ expression in the area postrema of total sleep deprived rats by TOF-SIMS Analysis

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ABSTRACT

Area postrema (AP) is a circumventricular organ plays an important role in sodium homeostasis and cardiovascular regulation. Since sleep deficiency will cause cardiovascular dysfunction, the present study aims to determine whether sodium level would significantly alter in AP following total sleep deprivation (TSD). Sodium level was investigated in vivo by time-of-flight secondary ion mass spectrometry (TOF-SIMS). Clinical manifestation of cardiovascular function was demonstrated by mean arterial pressure (MAP) values. Results indicated that in normal rats, TOF-SIMS spectrum revealed a major peak of sodium ion counting as 5.61×10^5 at *m/z* 23. The sodium ions were homogeneous distributed in AP without specific localization. However, following TSD, the sodium intensity was extremely increased (6.73×10^5) and the signal for sodium image was strongly expressed throughout AP with definite spatial distribution. MAP of TSD rats is 138 ± 5 mmHg, which is significantly higher than that of normal ones (121 ± 3 mmHg). Regarding AP is an important area for sodium sensation and development of hypernatremic related sympatho-excitation; up-regulation of sodium expression following TSD suggests that high sodium level might over-activate AP, through complex neuronal networks involving in sympathetic regulation, which could lead to the formation of TSD relevant cardiovascular diseases.

Keywords: Sodium, Area postrema, Sleep deprivation, Cardiovascular function, TOF-SIMS

1.INTRODUCTION

With the coming of the industrialization, sleep deprivation is increasingly becoming a major public health issue and affects millions of people in many countries [1]. Chronic sleep deprivation would lead to reduced alertness and lowered the decision-making process, which could increase the risk of motor vehicle accidents or other occupational injuries [2]. Clinical study has indicated that subjects suffering from sleep deprivation would cause excitation of the sympathetic nervous system, which unavoidably leads to cardiovascular disturbances [3]. Our previous study also

demonstrated that sleep deprivation would depress the metabolic state of afferent neurons involved in sympathetic inhibition [4]. Based on this viewpoint, it is suggested that sleep deprivation may increase the cardiovascular risk as a result of enhanced sympathetic activities [5].

The area postrema (AP) is a circumventricular organ located in the dorsal surface of the caudal brainstem. During the past few decades, AP has been implicated to play an important role in the control of sodium homeostasis and sympathetic functions [6,7]. Previous studies have indicated that AP is widely participated as a functioning part of a complex neuronal pathway regulating sympathetic activities [8,9]. Electrophysiological reports also demonstrated that AP is endowed with numerous chemosensitive neurons that may serve as sodium enteroceptors and act as an essential site for cardiovascular regulation [10,11]. It has been indicated that increased plasma sodium levels would contribute to hypertension in which the pressor effects may attribute to the over-activation of sodium sensitive neurons within the AP [12,13]. Through a complex neuronal network interlinking AP with other brainstem sympathetic regions, the sodium-dependent activation of AP is hence considered to play a vital role in the development of cardiovascular diseases [14].

However, although maintaining sodium expression in AP at the appropriate level may be crucial in homeostatic regulation of sympathetic activity, it is still unclear whether sodium expression in AP would significantly alter under sleep deprivation, a stressful condition with sympathetic activation. Moreover, whether the probable change of sodium expression in AP is positively correlated with the manifestation of clinical dysfunction is also remained to be explored. As an attempt to answer this question, and to provide sodium expression in a sensitive molecular imaging level, we decided to investigate the in vivo sodium expression by the use of time-of flight secondary ion mass spectrometry (TOF-SIMS). In addition, in order to examine the clinical function responding to the changes in sodium expression, the mean arterial pressure (MAP) measurement was further processed in the present study.

2.EXPERIMENTAL

Treatments and experimental animals

Adult male Wistar rats (n = 36, weighing 200 ~ 250 g) obtained from the Laboratory Animal Center of the National Taiwan University were used in this study. The experimental animals were divided equally into two groups. Rats in the first group were subjected to TSD for five days (TSD group), while those in the second group were housed in the TSD apparatus but were permitted to sleep (normal group). TSD was performed by the disc-on-water (DOW) method as described in our previous study [4]. Briefly, the apparatus was composed of two rectangular clear plastic chambers (60 × 20 × 60 cm in each) placing side by side. A single plastic disc (40 cm in diameter) serving as the rat-carrying platform was built in the lower quarter of the two chambers. Beneath the disc and extending to the chamber walls was a rectangular tray that was filled with water to a depth of 5 cm. An electric motor was set to run the rat-carrying disc at a moderate speed of 3.5 rev/min for 5 days; it was considered as a complete cycle for 8 s with an interval for 15 s. Sleep deprivation

depends on the rat's aversion to water, since rats rarely entered the water spontaneously. As sleep deprivation begins, rats in the TSD group placing on the disc had to keep awake and walk against the direction of disc rotation to avoid being forced into the water. For normal group, rats were allowed to sleep wherein no disc movement was initiated. All experimental animals were exposed to an automatically regulated light:dark cycle of 12:12 at a constant temperature of 25℃ ± 1℃. Food and water were made available through grids placed on top of the chambers. In the care and handling of all experimental animals, the Guide for the Care and Use of Laboratory Animals (1985) as stated in the United States NIH guidelines (NIH publication No. 86-23) were followed. All the experiments with sleep deprivation were also approved by the Laboratory Animal Center Authorities of the Chung Shan Medical University.

Haemodynamic measurement

After five days of TSD, all rats were anesthetized by intraperitoneal injection of Inactin (100 mg/kg body wt) and placed on a heating pad to maintain body temperature at 37 ℃. The mean arterial pressure (MAP) was then measured by inserting a polyethylene catheter (PE-10) into the right femoral artery. The haemodynamic variables of the MAP were measured using a pressure transducer (model P23 ID; Gould, Glen Burnie, MD) connected to a polygraph and were recorded by a thermal recorder (7758 B Sytsem; Hewlett-Packard, Palo Alto, CA).

Perfusion and tissue preparation

For TOF-SIMS analytical study, rats were deeply anesthetized with Inactin (100 mg/kg body weight, i.p.) and perfused transcardially with 0.9 % saline followed by 300 ml of 4 % paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The lower brainstem containing the AP were then removed under a surgical microscope (ZEISS DV4) and immersed in 30 % sucrose buffer at 4 ℃ overnight for cryoprotection. Serial 30 μm thick sections of the tissue were cut transversely with a cryostat on the following day and the collected sections were attached to the silica wafers (1 cm x 1 cm).

TOF-SIMS analysis

TOF-SIMS analysis was carried out on a TOF-SIMS IV instrument (ION-TOF GmbH, Münster, Germany). The gallium (Ga^+) ion gun operated at 25 kV was used as the primary ion source (1 pA pulse current) for experiments conducted in this study. The Ga^+ primary ion beam was scanned over an area of 100 μm^2. Positive secondary ions flying through a reflectron mass spectrometer were detected with a microchannel plate assembly operating at 10 kV post-acceleration. Mass calibration of the ion spectrum was achieved by using a set of mass peaks like *m/z* 15 (CH_3^+), 41 ($C_3H_5^+$) and 69 (Ga^+). The ions related to Na^+ (*m/z* 23) were used to identify and evaluate the molecular image of sodium expression.

3.RESULTS AND DISCUSSION

Mean arterial pressure analysis

The changes of MAP of both experimental groups were summarized in Fig 1. In normal rats, the MAP was estimated to be 121 ± 3 mmHg. However, in rats suffering from TSD, the MAP was significantly increased to nearly 138 ± 5 mmHg. These results suggested that TSD would exert a pressor effect on blood pressure, which might be elicited by potentiation of the sympathetic activity.

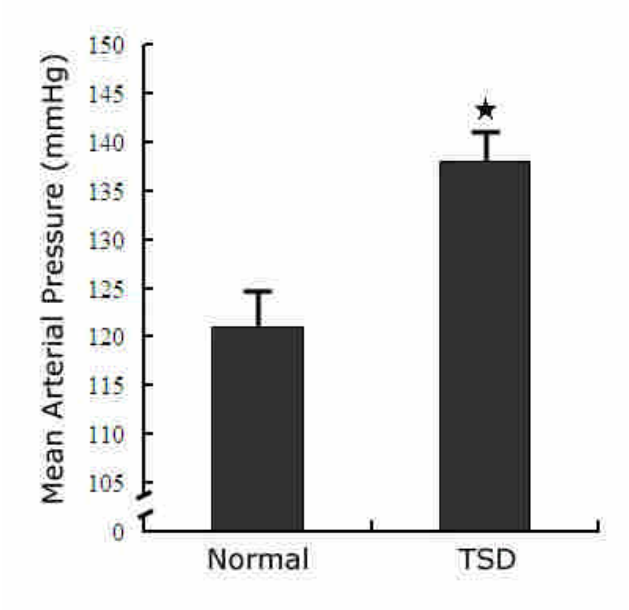

Fig. 1 Histogram showing the mean arterial pressure (MAP) in the normal and TSD rats. Note that the MAP is significantly higher in TSD rats than that of normal ones. ★ $p<0.05$, Student's *t*-test.

TOF-SIMS mass spectra

Fig. 2 shows the TOF-SIMS positive ion mass spectra in the *m/z* of 23 that reflects the intensity distribution of sodium ion in the AP of both normal and TSD rats. The analytic region is 1 mm above the obex with an area of 500 x 500 μm^2. The typical positive ion spectrum revealed that the intensity for major peak of sodium in AP was counted to be 5.61×10^5 in normal rats (Fig. 2A). However, following 5 days of TSD, the spectrum for sodium ion intensity in AP was drastically increased to 6.73×10^5 (Fig. 2B). It is worthy to note that the spectral intensities for calibrated ions (e.g. *m/z* 15) were similar in both normal and TSD groups, suggested that the intensity change of sodium ion observed in current experimental paradigm is a significant and specific effect. It has been reported that AP has a high number of sodium sensitive chemoreceptors that may be involved in sodium homeostasis and cardiovascular regulations [10,11]. Previous report also demonstrated that increase sodium intake would contribute to the development of hypertension in which the pressor effect was proposed to be arisen from over-activation of sodium sensitive chemoreceptors within AP [12,13].

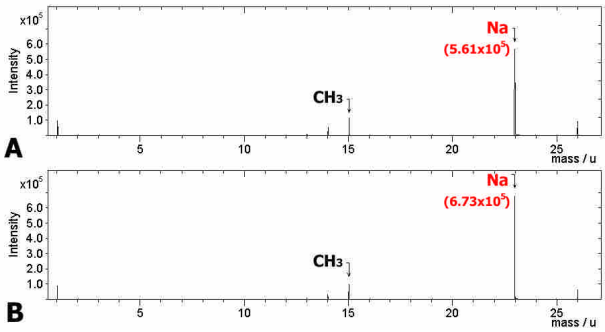

Fig. 2 TOF-SIMS positive ion spectrum showing the sodium ion intensity in the area postrema (AP) of normal (A) and TSD (B) rats. Note that the specific signal from sodium ion (*m/z* = 23) was markedly increased in the TSD rats (B) as compared with that of normal ones (A). Also note that the intensity of calibrated signals (e.g. *m/z* 15) was similar in both normal and TSD groups, suggested that the up-regulation of sodium expression in TSD rats was a specific effect.

As one of the integrative sites for cardiovascular regulation, AP is shown to serve as a functional part of a complex neuronal networks regulating sympathetic activity [8,9]. It is indicated that AP sends projections to numerous brainstem regions that have been identified to play important roles in enhancing sympathetic activity such as rostral ventrolateral medulla (RVLM) and parabrachial nucleus [8,9]. Through the close interlinking between AP and these sympatho-excitatory areas, increased sodium levels in the AP, as seen in our current TSD paradigm, would

over-activate sodium responsive neurons and consequently lead to sympathetic associated cardiovascular dysfunctions [15,16].

TOF-SIMS molecular image

TOF-SIMS positive ion image has revealed that in normal rats, the sodium ions were homogeneous distributed throughout the AP without any specific localization (Fig. 3A). The AP profile is clear visible suggested that our tissue section prepared for TOF-SIMS is quite fit. However, in TSD animals, the sodium ions were strongly expressed in AP with significant spatial distribution (Fig. 3B). Some sodium ion clusters with round profiles were found to be presented in distinct regions within AP, which might well mark the locations of over-activated sodium sensitive neurons within this area (Fig. 3B). It is suggested that elevated plasma sodium levels may play an important role in pathogenesis of hypernatremic related hypertension [12,13]. Since AP is the major site for the control of sodium homeostasis [10], up-regulation of sodium expression in AP would evidently elicit cardiovascular changes by means of AP-mediated sympathetic activity. In the present study, we further observed a significantly higher MAP value in TSD rats with apparent sodium increase in AP, which clearly demonstrate a possible mechanism of hypernatremic relevant cardiovascular diseases. However, the present study did not detect the potential changes of other brainstem nuclei or directly assess the sympathetic activity. As any cardiovascular changes is rose from the integration of a complex neuronal network, examining other neurochemical alteration in brainstem sympathetic regions or evaluating the sympathetic activity should be performed in the further study.

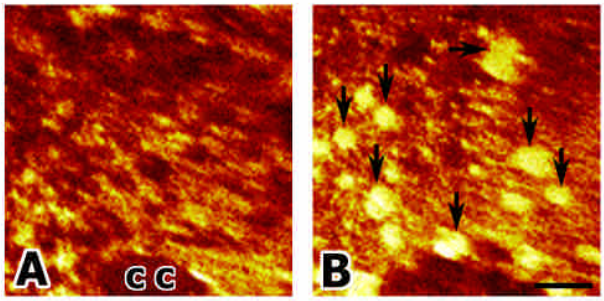

Fig. 3 TOF-SIMS positive ion image showing the sodium ion expression in the area postrema (AP) of normal (A) and TSD (B) rats. The ion intensities in the molecular image are represented by a color scale with dark colors representing low intensities and bright colors representing high intensities. Note that in normal rats, the sodium ions were homogenous distributed in AP (A). However, following TSD, the sodium ions were strongly expressed in AP and were aggregated into significant clusters (arrows) with distinct localization within this region (B). cc: central canal; Scale bar = 100 μm.

4. CONCLUSIONS

The present study is the first report employing TOF-SIMS analysis to provide molecular imaging evidence that sodium ions would drastically up-regulate in the AP of total sleep deprived rats. The clinical significance of enhanced sodium expression is functionally reflected by the elevated mean arterial pressure, a typical feature of sympathetic activation. To the extent of our knowledge, the present study has addressed for the first time that TOF-SIMS could be served as a useful tool for imaging the ion level and distribution at the *in vivo* condition. Since TOF-SIMS has become one of the most sensitive analytical techniques used in current molecular cell biology, advanced use of TOF-SIMS in exploring other sympathetic related regions following TSD will be greatly

helpful in increasing our knowledge of TSD relevant cardiovascular diseases.

5.ACKNOWLEDGEMENT

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