

# Arterial Pressure and Heart Rate Increase during REM Sleep in Adenosine A<sub>2A</sub>-Receptor Knockout Mice, but not in Wild-Type Mice

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Rapid eye movement (REM)-sleep related changes in arterial pressure (AP) and heart rate (HR) were observed in homozygous and heterozygous adenosine A<sub>2A</sub> receptor (A<sub>2A</sub>AR) knockout (KO) mice, and the corresponding wild-type mice. During REM sleep, the mean AP (MAP) and HR were clearly increased in the homozygous A<sub>2A</sub>AR KO mice, while, in the wild-type mice, they were decreased or maintained at the same level. Neither homozygous nor heterozygous A<sub>2A</sub>AR KO mice showed significant difference in diurnal pattern and the hourly values of MAP and HR compared to the wild-type mice. From these findings, it is likely that the adenosine A<sub>2A</sub>AR is involved in autonomic regulation during REM sleep.

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## INTRODUCTION

Rapid eye movement (REM) sleep has a particular characteristic in terms of cardiovascular control (Parmeggiani, 2000; Sei and Morita, 1999). During non-REM sleep, autonomic functions such as arterial pressure (AP), heart rate (HR), and respiration are quite stable and show constant values. On the other hand, during REM sleep, significant fluctuations in autonomic functions are observable. Physiological homeostatic control appears to be suppressed under REM sleep condition, representing an increased risk due to marked cardiovascular instability (Parmeggiani, 2000). Clinically, obstructive sleep apnea is very much a REM-sleep related disease, with obstructive apneas occurring more frequently for a longer duration, and with more desaturation during REM sleep than non-REM sleep (Arens and Marcus, 2004; Goh *et al*, 2000). However, the central mechanism for this autonomic change associated with REM sleep remains unclear.

Patients with panic disorder and/or post-traumatic stress disorder (PTSD) have an altered HR variability during REM sleep, determined by frequency analysis of the time course changes in the electrocardiogram R–R interval (Mellman *et al*, 2004; Nishith *et al*, 2003; Sloan *et al*, 1999). Such patients show a higher sympathetic component in HR variability during this sleep period. Furthermore, sympathetic predominance during REM sleep decreases in the case of PTSD patients who have been successfully treated by behavioral therapy (Nishith *et al*, 2003). Polymorphisms in the adenosine A<sub>2A</sub> receptor (A<sub>2A</sub>AR) has recently been reported to be linked to the panic disorder in human (Deckert *et al*, 1998; Hamilton *et al*, 2004), and are also associated with anxiogenic responses to caffeine (Alsene *et al*, 2003). Based on the above genetic data, the possibility that adenosine A<sub>2A</sub>AR may be involved in the change in autonomic function during REM sleep cannot be excluded. The A<sub>2A</sub>AR expression is highest in striatum, nucleus accumbens and olfactory tubercle, and also presents in the heart, kidney, lung, liver, and platelets. The A<sub>2A</sub>AR is thought to play a variety of roles in physiological responses and pathological conditions. Especially, antagonistic interaction between A<sub>2A</sub>AR and dopamine D<sub>2</sub> receptor (D<sub>2</sub>R), relating to motor function or psychotic state, has been well documented (Moreau and Huber, 1999).

In this study, we observed the changes in AP and HR under normal sleep/wake conditions in adenosine A<sub>2A</sub>AR knockout (KO) mice in order to clarify the role of A<sub>2A</sub>AR in REM-sleep related cardiovascular function.

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## MATERIALS AND METHODS

A2AR KO mice were derived from animals supplied by Chen *et al* (1999) (Harvard Medical School) and the generation and characterization of these mice has been described previously.

Homozygous ( $A2AR^{-/-}$ ;  $n=8$ ) and heterozygous ( $A2AR^{+/-}$ ;  $n=6$ ) A2AR KO and the corresponding wild-type ( $A2AR^{+/+}$ ;  $n=7$ ) mice (10–15 weeks of age and weighing 26–30 g) were used in this study. They were maintained under 12:12 h light:dark cycle (lights on between 09 00 and 21 00) at ambient temperature ( $24 \pm 1^\circ\text{C}$ ). Food and water were available *ad lib*. All experiments were carried out according to the guidelines for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

Under ketamine and xylazine anesthesia (100 and 25 mg  $\text{kg}^{-1}$ , respectively), the tip of a catheter of a telemetric device (TA11PA-C20; Data Sciences Int., USA) was inserted into the aortic arch via the left carotid artery, with the telemeter body positioned subcutaneously on the right flank. In addition to the telemetric device, stainless-steel miniature screw electrodes were implanted in the skull to record an electroencephalogram (EEG), and Teflon coated stainless-steel wires were implanted in the neck muscles on both sides to record an electromyogram (EMG). After the surgery, the mice were housed individually in square plastic cages (length and width 30 cm, depth 35 cm) in a sound-proofed recording room. The mice were connected by flexible cables to a polygraph and a computer-assisted data acquisition system (CED 1401 data processor, Cambridge Electronic Design, UK). At least 10 days after the surgery, polygraphic recordings for AP, EEG, and EMG were performed continuously for a 24-h period.

Offline sleep scoring was carried out on a computer screen by a visual assessment of EEG and EMG activity. A vigilance state in each 5-s epoch was classified as wake, non-REM sleep and REM sleep. Non-REM sleep was characterized by a continuous slow high-voltage EEG and low-level EMG activity. REM sleep was characterized by a low-voltage

EEG with continuous theta waves and a total suppression of EMG. The mean AP (MAP) was calculated as the average of the digitized AP signal in consecutive 1-s epochs, and HR was detected from the AP signal. For observation of the diurnal features of MAP and HR, the MAP and HR were averaged hourly for 24 h for each animal, and then averaged for each group.

In order to observe the changes in MAP and HR in the transition from NREM to REM sleep and from REM sleep to wake, the MAP and HR values were averaged in every 5-s periods from 1 min before to 1 min after the onset or end of REM sleep. REM sleep in which the duration was more than 1 min was used for the analysis. MAP and HR were expressed as variations from the baseline values determined as averages of MAP or HR during 30-s periods starting at 1-min before the onset of REM sleep. The data were averaged for each mouse, and then averaged for each group.

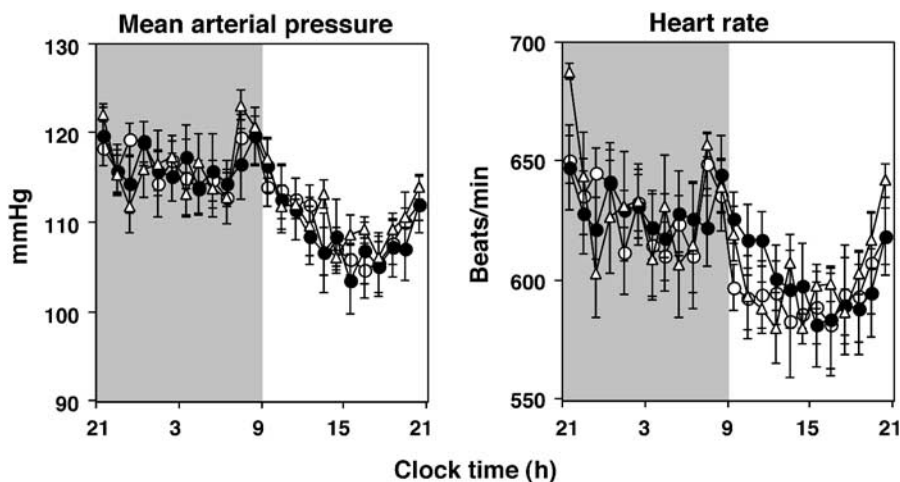
In the  $A2AR^{+/+}$  and  $A2AR^{-/-}$  mice, a 512-point fast Fourier Transform analysis was performed on the artifact-free EEG during REM sleep episodes. The averaged EEG spectrum and peak power in each animal were averaged for each group.

The results are expressed as means  $\pm$  SEM. Time course changes in AP and HR were assessed by two-way analysis of variance (ANOVA). Student's *t*-test was used for the comparison in the EEG peak power. Statistical significance was accepted at the conventional  $p < 0.05$  level.

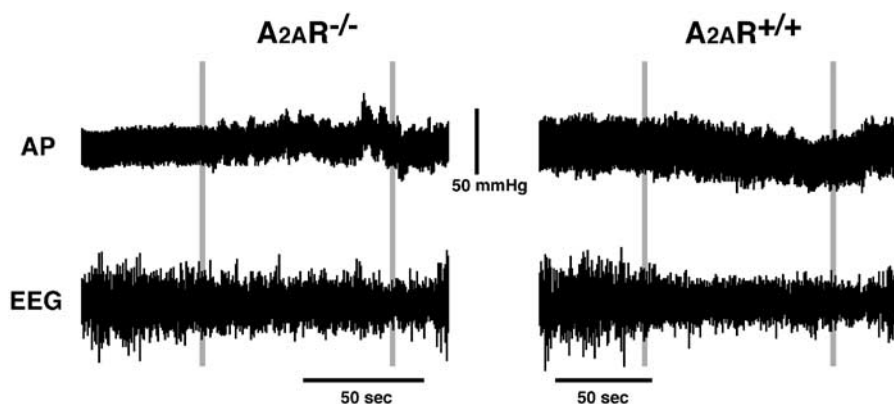
## RESULTS

All groups of mice showed a clearcut diurnal rhythm in both AP and HR, being higher during the dark (active) period and lower during the light (resting) period. There was no significant difference in the diurnal pattern and hourly values of MAP and HR among the three groups as shown in Figure 1.

During REM sleep, a drastic difference among the three genetic types of mice was noted. Figure 2 shows examples of



**Figure 1** Diurnal profiles (mean  $\pm$  SEM) in mean arterial pressure and heart rate under 12–12 h light/dark conditions in wild type ( $n=7$ , open circles), heterozygous ( $n=6$ , open triangle) and homozygous ( $n=8$ , closed circles) adenosine A2A receptor knockout mice. The gray zone in each figure indicates a dark period. Mean arterial pressure: [group]  $F(2, 18) = 0.044\text{NS}$ , [time]  $F(23, 414) = 17.734$   $p < 0.0001$ , [interaction]  $F(46, 414) = 0.987\text{NS}$ . Heart rate: [group]  $F(2, 18) = 0.041\text{NS}$ , [time]  $F(23, 414) = 9.343$   $p < 0.0001$ , [interaction]  $F(46, 414) = 1.052\text{NS}$ .



**Figure 2** Representative examples of REM sleep with arterial pressure in homozygous adenosine A2A receptor knockout mice ( $A2AR^{-/-}$ , left) and wild-type mice ( $A2AR^{+/+}$ , right). Vertical lines indicate the onset and end of REM sleep. AP: arterial pressure. EEG: electroencephalogram.

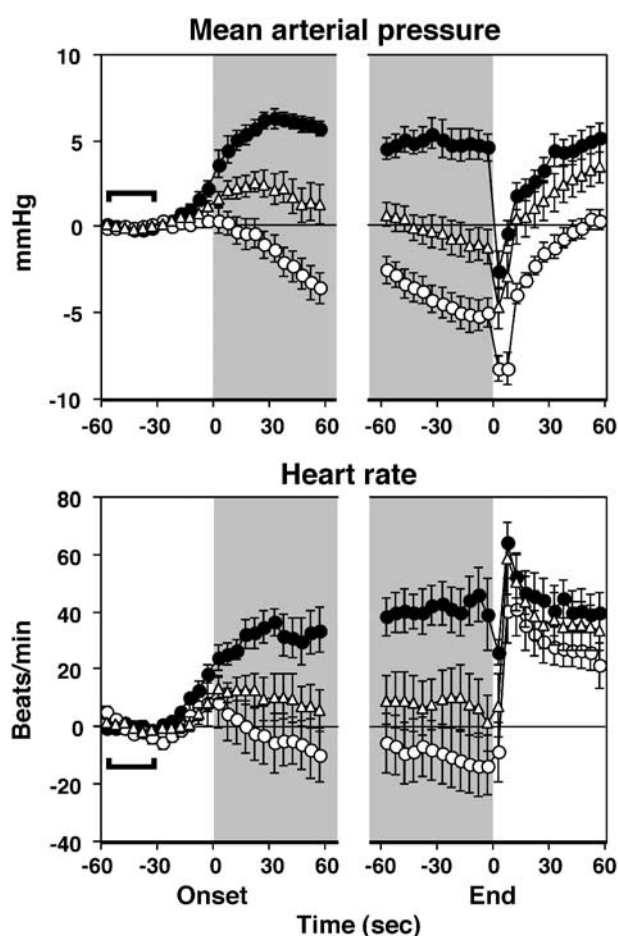
the AP profiles during REM sleep in  $A2AR^{-/-}$  and  $A2AR^{+/+}$  mice. In the  $A2AR^{+/+}$  mice, the AP was decreased during REM sleep, while, in the  $A2AR^{-/-}$  mice, a large increase of AP was noted during REM sleep. As shown in Figure 3, significant differences among three groups for the averaged MAP and HR were found. Both MAP and HR in the  $A2AR^{-/-}$  mice significantly increased from the baseline level (that is in non-REM sleep) throughout REM sleep. The  $A2AR^{+/+}$  mice showed intermediate levels for AP and HR for the  $A2AR^{-/-}$  and  $A2AR^{+/+}$  mice throughout the period of REM sleep. At the end of REM sleep, sudden drops of both AP and HR were observed in the  $A2AR^{-/-}$  mice, while, in the  $A2AR^{+/+}$  and  $A2AR^{+/+}$  mice, HR did not decrease.

The EEG spectrum for the  $A2AR^{-/-}$  and  $A2AR^{+/+}$  mice during REM sleep were quite similar (Figure 4), showing clear theta activity around 7–8 Hz. The peak power of the spectrum, that is the peak theta power, was not different between the groups ( $t = 0.629$ ,  $p = 0.543$ ).

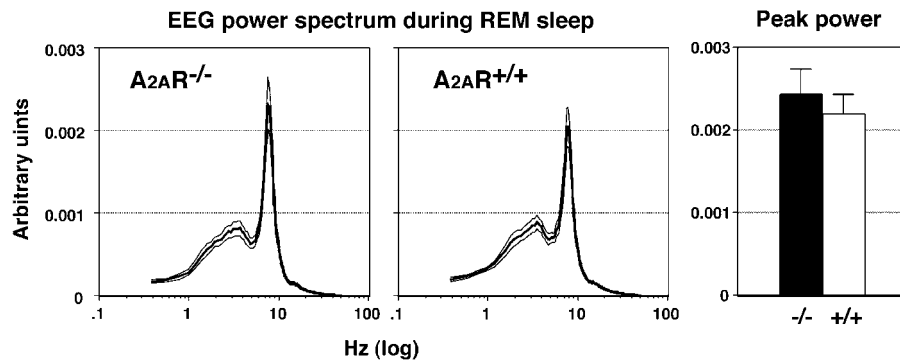
## DISCUSSION

The findings herein show that adenosine A2AR is closely involved in autonomic functions during REM sleep. A lack of A2AR appears to be one of the factors that causes an increase in AP and HR during REM sleep without any change in the diurnal pattern or the hourly averaged values.

It is well known that, in the brain, A2AR is particularly abundant in the nucleus accumbens and striatum (Rosin *et al*, 1998). The receptors in this area are involved, not only in motor function, but also anxiety, stress response, or motivation (Ledent *et al*, 1997; Moreau and Huber, 1999). It is noteworthy that data from clinical and behavioral pharmacological studies have implicated A2AR in anxiety behaviors, while genetic studies have suggested that A2AR is associated with panic disorder (Alsene *et al*, 2003; Hamilton *et al*, 2004; Moreau and Huber, 1999). The normal cat shows an increase in AP and HR during REM sleep as seen in the normal rat (Sei *et al*, 1989; Sei *et al*, 1994). On the other hand, a cat whose brainstem has been transected at the ponto-mesencephalic junction (decerebrated cat) shows a REM-sleep like state with muscle atonia and REM, and, during this state, it does not show any increase in AP and HR (Kanamori *et al*, 1995). Therefore, the descending command for increasing AP and HR during REM sleep is



**Figure 3** Time course changes (mean  $\pm$  SEM) in mean arterial pressure (MAP) and heart rate (HR) over the period 1 min before and 1 min after the onset or end of rapid eye movement (REM) sleep in wild type ( $n = 6$ , open circles), heterozygous ( $n = 6$ , open triangle), and homozygous ( $n = 6$ , closed circles) adenosine A2A receptor knockout mice. The gray zone in each figure indicates REM sleep. Data are expressed as relative values from the baseline obtained from the 30 s period indicated by horizontal black bars. MAP onset: [group]  $F(2, 15) = 20.773$   $p < 0.0001$ , [time]  $F(23, 345) = 19.444$   $p < 0.0001$ , [interaction]  $F(46, 345) = 21.614$   $p < 0.0001$ . MAP end: [group]  $F(2, 15) = 20.847$   $p < 0.0001$ , [time]  $F(23, 345) = 73.844$   $p < 0.0001$ , [interaction]  $F(46, 345) = 9.258$   $p < 0.0001$ . HR onset: [group]  $F(2, 15) = 5.110$   $p < 0.05$ , [time]  $F(23, 345) = 8.728$   $p < 0.0001$ , [interaction]  $F(46, 345) = 5.105$   $p < 0.0001$ . HR end: [group]  $F(2, 15) = 4.222$   $p < 0.05$ , [time]  $F(23, 345) = 47.913$   $p < 0.0001$ , [interaction]  $F(46, 345) = 7.691$   $p < 0.0001$ .



**Figure 4** Averaged EEG power spectrum (mean ± SEM) during REM sleep in homozygous adenosine A2A-receptor knockout mice (A2AR<sup>-/-</sup>,  $n = 6$ ) wild-type (A2AR<sup>+/+</sup>,  $n = 6$ ). Comparison of the peak power in the EEG spectrum (mean ± SEM) is presented in right bar graph. No significant difference was found.

thought to originate in the forebrain structure. The neural mechanisms of psychological factors responsible for higher forebrain function to be affected by A2AR may be involved in autonomic functions during REM sleep. A psychological dysfunction such as panic disorder or PTSD and autonomic dysregulation during REM sleep may be linked through the A2AR function. A local injection of an A2AR antagonist into the basal ganglia, especially the nucleus accumbens, of wild-type mice would provide additional information concerning the sleep-related role of A2AR in the basal ganglia.

We previously reported that dopaminergic neurons are involved in the REM-sleep related cardiovascular control in the rat (Sei *et al*, 1999a). In the rat, under normal condition, AP and HR increase actively during REM sleep differently from the A2AR<sup>+/+</sup> (wild-type) mice used in this study. When midbrain-accumbens dopaminergic neurons (A10) are damaged by the injection of a catecholamine neurotoxin (6-hydroxydopamine), the increase in AP and HR during REM sleep is significantly suppressed, suggesting that the dopaminergic neurons activate the increase of AP and HR during REM sleep. Since A2AR is thought to have an antagonistic interaction with D2R in the striatum and accumbens (Kase, 2001; Moreau and Huber, 1999), the lack of A2AR may cause the disinhibition of the function of the D2R, thus inducing an increase in AP and HR during REM sleep. In many strains of mice, a decrease in AP and HR during REM sleep has been reported under normal conditions (Campen *et al*, 2002; Schaub *et al*, 1998). Our results for the wild-type mice used in the present study are consistent with previous reports. In normal mice, the power balance between the A2AR and D2R might be different from that in the normal rats. The administration of a D2R antagonist to the A2AR KO mice would provide additional evidence for this.

On the other hand, it has been proposed that A2AR in the nucleus tractus solitarius (NTS) is an important contributor to the integration of cardiovascular afferent information from the baroreceptor and/or chemoreceptor (Moreau and Huber, 1999; Phillis *et al*, 1997), and to be critically involved in cardiovascular reflex control. Although the density of A2AR in the NTS is lower compared to the striatum and accumbens, blockade of the A2AR in this area decreases the sensitivity of the baroreflex control of HR (Mosqueda-Garcia *et al*, 1989; Thomas *et al*, 2000). In fact, in the A2AR KO mice used in this study, we found the larger standard

deviation for AP during the 6-h period in the middle of dark (waking with no REM-sleep) phase (A2AR<sup>-/-</sup>:  $9.9 \pm 0.7$  ( $n = 6$ ), A2AR<sup>+/+</sup>:  $7.5 \pm 0.4$  ( $n = 6$ ),  $p < 0.05$  by Student's *t*-test). We previously reported that sino-aortic denervation, which causes the dysfunction of the baroreflex control, induces the augmentation of the increase in AP and HR during REM sleep (Sei *et al*, 1999b). Therefore, it is possible that A2AR KO mice have a lowered function of the baroreflex control and subsequently show an increased instability in AP and HR during REM sleep. Conversely, it is possible that the A2AR has an important role in stabilizing the cardiovascular system.

Furthermore, A2AR mRNA is expressed not only in central nervous system but also in peripheral arterial chemoreceptor. It is noteworthy that an antagonistic interaction between adenosine and dopamine in the chemoreceptive function has been reported (Gauda, 2002; Gauda *et al*, 2000). Therefore, it is also possible that the change in chemoreceptive function caused by A2AR KO induces an alteration in cardiovascular control during REM sleep.

A phasic surge of AP often appears during REM sleep, accompanied by the activation of EEG theta rhythm in the rat (Sei and Morita, 1996). In this study, no significant difference in EEG spectrum and peak theta power during REM sleep between A2AR<sup>-/-</sup> and A2AR<sup>+/+</sup> mice was found. Therefore, the MAP increase during REM sleep observed in our A2AR KO mice appears not to be caused by a change in phasic but, rather, by a tonic phenomenon.

In conclusion, a lack of A2AR induces an increase in AP and HR during REM sleep. As Campen *et al* (2002) proposed, the findings herein clearly show that genetic factors play an important role in autonomic functions during REM sleep. A study of a relationship between adenosine A2AR function or its gene polymorphism and the pathophysiological mechanism in disorders related to autonomic dysregulation during REM sleep, including sleep apnea syndrome or a cardiovascular ischemic accident, would be of interest.

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