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Riluzole suppresses post-sigh, but not spontaneous apnoeas during sleep in rats

Miodrag Radulovacki, Sasa Pavlovic, Andrei Rakic, Marika Janelidze, Lisa Shermulis and David W. Carley

Abstract

We conducted this experiment to determine the role of glutamate in the mechanism of sleep apnoeas by administering riluzole, a glutamate release inhibitor, to freely moving rats in which sleep-related apnoeas are physiological phenomena. Adult Sprague-Dawley rats were implanted with electrodes for electroencephalogram (EEG) and electromyogram (EMG) recording to monitor sleep and were placed inside a single-chamber plethysmograph to monitor respiration. Sleep and respiration were recorded for 6 h following intraperitoneal administration of 0.5, 5.0 and 10.0 mg kg^{-1} riluzole. Riluzole dose-dependently suppressed post-sigh apnoeas during rapid eye movement (REM) sleep but had no effect on sleep-related spontaneous apnoeas. The drug (5.0 and 10.0 mg kg^{-1}) also dose-dependently reduced wakefulness and increased sleep. It appears that glutamate, an excitatory neurotransmitter, plays a role in the genesis of the post-sigh apnoeas during REM sleep.

Department of Pharmacology, University of Illinois at Chicago, Chicago, IL, USA

Miodrag Radulovacki, Sasa Pavlovic, Andrei Rakic, Lisa Shermulis, David W. Carley

Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, USA

Marika Janelidze, David W. Carley

Department of Medicine, University of Illinois at Chicago, Chicago, IL, USA

Miodrag Radulovacki, David W. Carley

Correspondence: M. Radulovacki, Department of Pharmacology, M/C 868, University of Illinois at Chicago, 901 S. Wolcott Avenue, Chicago, IL 60612, USA.
E-mail: mradulo@uic.edu

Introduction

Glutamate, an excitatory neurotransmitter, participates in the mechanism of reflex apnoeas which are critical for protecting the airways and lungs from aspiration of water or food. In anaesthetized rats, microinjections of glutamate into the intertrigeminal region (ITR) elicited reflex apnoeas (Chamberlin & Saper 1998) whereas unilateral injections of the ionotropic glutamate receptor antagonist AP5 into the Kölliker-Fuse nucleus (KF), an area next to the ITR, led to a significant blockade of the ethmoidal nerve-evoked respiratory depression (Dutschmann & Herbert 1998). Although the central mechanisms responsible for eliciting sensory-induced apnoea are not well understood, these studies point to the role of glutamate in the ITR and KF as an important neurotransmitter in producing apnoeas and in mediating apnoea-producing reflexes.

Our studies on sleep-related apnoeas in freely moving rats indicated a role of peripheral serotonin in the genesis of rapid-eye-movement (REM) sleep-related spontaneous apnoeas (Radulovacki et al 1998). Thus, intraperitoneal administration of serotonin increased REM sleep-related spontaneous but not post-sigh apnoeas; an effect completely abolished by pretreatment with 5-HT_3 receptor antagonists (Carley & Radulovacki 1999a). Since serotonin does not cross the blood–brain barrier, this effect was attributed to peripheral origin. In view of recent experiments regarding the effects of glutamate on reflex apnoeas in the pons, we employed intraperitoneal administration of riluzole, which inhibits glutamate

release (Doble 1997) by influencing different pre- and postsynaptic processes of glutamate transmission (Bryson et al 1996) and which crosses the blood–brain barrier (Manz 1996), to observe whether inhibition of central glutamate release affects sleep-related apnoeas in freely behaving rats.

Materials and Methods

Drugs

Riluzole (Rilutek; Rhone-Poulenc Rorer) tablets (50 mg) were obtained from the University of Illinois Medical Center Pharmacy and dissolved in physiologic saline.

Animals

Ten adult male Sprague-Dawley rats (300 g) were maintained on a 12-hour light–dark cycle for one week, housed in individual cages and given free access to food and water. After 1 week of adaptation, rats were subjected to surgical procedures which will be briefly described here. All procedures and protocols conformed to the Helsinki Accords and the policies of the American Physiological Society regarding animal experimentation, and were approved by the Animal Care Committee of the University of Illinois at Chicago.

Experimental procedure

Rats were anaesthetized for the implantation of cortical electrodes for electroencephalogram (EEG) recording, and neck-muscle electrodes for electromyogram (EMG) recording, using a mixture of ketamine (Vetalar 80 mg kg^{-1}) and xylazine (5 mg kg^{-1}) as we have described previously (Carley & Radulovacki 1999a). After the surgery, rats were allowed a one-week recovery period before being used in the study. Each rat was recorded on four occasions, in random order, after intraperitoneal injection (1 mL kg^{-1}) of either saline (control) or 0.5, 5.0 or 10.0 mg kg^{-1} riluzole. Polygraphic recordings for an individual rat were separated by at least 3 days. There were 10 rats in each treatment group.

Respiration was recorded by placing each rat, unrestrained, inside a single-chamber plethysmograph (PLYUNIR/U; Buxco Electronics, Sharon, CT; 6 inches wide × 10 inches long × 6 inches high) ventilated with a bias flow of fresh air at a rate of 2 L min^{-1}. A cable plugged onto the rat's connector and passed through a sealed port carried the bioelectrical activity from the head. Respiration, EEG and EMG were displayed on a video monitor and simultaneously digitized 100 times per second and stored on computer disk (Experimenter's Workbench; Datawave Technologies, Longmont, CO). Sleep and waking states were assessed using the biparietal EEG and nuchal EMG signals on 10-s epochs as described by Benington et al (1994). This software discriminated wakefulness (W) as a high-frequency, low-amplitude EEG with a concomitant high EMG tone; non-rapid eye movement (NREM) sleep by increased spindle and theta activity together with decreased EMG tone, and rapid eye movement (REM) sleep by a low ratio of a delta-to-theta activity and an absence of EMG tone.

As in previous investigations (Monti et al 1995; Carley et al 1996; Radulovacki et al 1996), sleep apnoeas, defined as cessation of respiratory effort for at least 2.5 s, were scored for each recording session, and were associated with the stage in which they occurred: NREM or REM sleep. The duration requirement of 2.5 s represented at least two missed breaths, and was therefore analogous to a 10-s apnoea duration requirement in humans. The events detected represented central apnoeas because decreased ventilation associated with obstructed or occluded airways would generate an increased plethysmographic signal, rather than a pause. As in previous reports, apnoeas were observed to occur as pauses between breaths (spontaneous apnoeas) or immediately following a sigh (post-sigh apnoeas). Sighs were identified as tidal volumes at least 150% greater than the overall mean tidal volume for each recording (Monti et al 1995). Apnoea index, defined as apnoeas per hour in a stage, were separately determined for NREM and REM sleep.

Statistical analysis

The effects of sleep stage (NREM vs REM) and injection (control vs three active injections) on apnoea indexes, respiratory rate, and inspired minute ventilation were tested using analysis of variance with repeated measures. Multiple comparisons were controlled using Fisher's protected least-significance difference (PLSD).

Results

Table 1 presents the results of the two-way analysis of variance for the main effects of behavioural state (sleep stage) and riluzole dose on apnoea indexes, respiratory

Table 1 Significance of sleep-stage and riluzole-dose factors on respiration in rats (analysis of variance).

	Spontaneous apnoea index	Post-sigh apnoea index	Respiratory rate	Minute ventilation
Sleep stage				
F	25.12	27.74	5.00	5.54
d.f.	1[a]	1[a]	2[a]	2[a]
P	0.0007	0.0005	0.0001	0.004
Dose				
F	0.07	3.45	0.63	0.85
d.f.	3	3	3	3
P	0.98	0.03	0.60	0.47

[a]Sleep stages included NREM and REM for analysis of apnoea indexes, resulting in 1 degree of freedom for the test of this factor; all three behavioural states (Wake, NREM, REM) were included in the tests of state on respiratory rate and minute ventilation, resulting in 2 degrees of freedom.

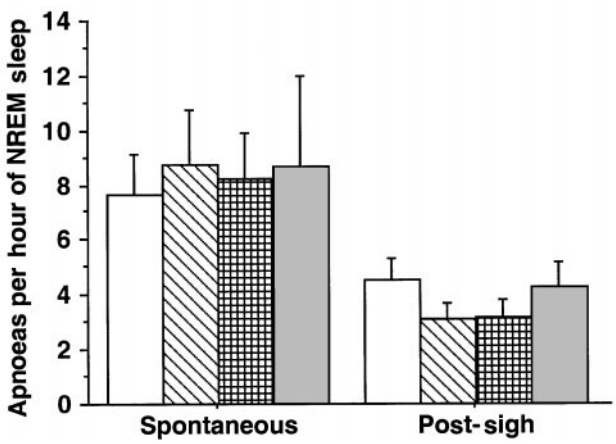

Figure 1 Effect of riluzole (0.5, 5.0 and 10.0 mg kg^{-1}) on the rate of spontaneous and post-sigh apnoeas per hour of NREM sleep in rats during 6 h polygraphic recordings. Saline control – open bar; riluzole 0.5 mg kg^{-1} – diagonal hatch bar; riluzole 5.0 mg kg^{-1} – cross hatch bar; riluzole 10.0 mg kg^{-1} – shaded bar. Each point represents mean ± s.e. for 10 rats.

rate and minute ventilation. Because this was a balanced repeated measures design, the analysis reflects pooled observations from 10 rats in each group. Table 1 demonstrates that fluctuations among behavioural states exerted a significant impact on each measure of respiratory pattern – including apnoea expression. Conversely, there was no significant influence of riluzole dose on minute ventilation, respiratory rate or spontaneous apnoea index. Riluzole did exert a significant impact on expression of post-sigh apnoeas ($P = 0.03$), an effect that was observed only during REM sleep ($F = 4.11$, d.f. $= 3$, $P = 0.01$ for interaction between sleep-stage and dose). These findings are elaborated in greater detail below.

Table 2 presents the quantitative effects of behavioural state and riluzole dose on respiratory rate and minute ventilation. As indicated in Table 1, riluzole dose had no impact on either of these respiratory pattern variables overall (from two-way analysis of variance) or in each individual behavioural state ($P > 0.20$ for each one-way analysis of variance for each variable). The significant influence of behavioural state observed under control (saline) conditions is lost ($P > 0.05$) on respiratory rate at the 10.0 mg kg^{-1} dose and on minute ventilation at all riluzole doses (Table 2).

Figure 1 shows the effects of riluzole (0.5, 5.0 and 10.0 mg kg^{-1}) on the rate of spontaneous and post-sigh apnoeas per hour of NREM sleep during the 6 h of polygraphic recording. Analysis of variance with repeated measures showed no significant effect of treatment over 6 h.

Table 2 Impact of sleep stage and riluzole dose on respiratory rate and minute ventilation in rats.

	Respiratory rate (min^{-1})				Minute ventilation (mL min^{-1})			
	Saline	Riluzole			Saline	Riluzole		
		0.5 mg kg^{-1}	5.0 mg kg^{-1}	10.0 mg kg^{-1}		0.5 mg kg^{-1}	5.0 mg kg^{-1}	10.0 mg kg^{-1}
Wakefulness	116.0 ± 5.8	114.6 ± 4.5	117.5 ± 6.5	106.4 ± 4.4	81.8 ± 0.8	81.0 ± .8	84.2 ± .7	83.4 ± .08
NREM	104.2 ± 2.9	103.0 ± 2.0	101.8 ± 1.8	101.8 ± 3.8	72.8 ± .7	73.6 ± .10	76.1 ± .6	82.6 ± .09
REM	113.9 ± 3.1	118.1 ± 2.6	114.6 ± 3.6	113.3 ± 3.7	77.70 ± .5	80.2 ± .9	81.8 ± .8	83.4 ± .06
P	0.02	0.007	0.03	0.19	0.01	0.06	0.10	0.99

Results are presented as mean ± s.e. Number of animals in each group was 10. No significant dose effects were observed on respiratory rate or minute ventilation overall or within any behavioural state.

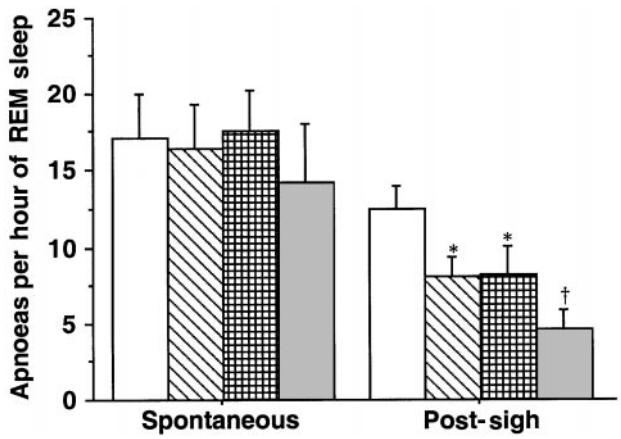

Figure 2 Effect of riluzole (0.5, 5.0 and 10.0 mg kg^{-1}) on the rate of spontaneous and post-sigh apnoea expression per hour of REM sleep in rats during 6 h polygraphic recordings. Saline control – open bar; riluzole 0.5 mg kg^{-1} – diagonal hatch bar; riluzole 5.0 mg kg^{-1} – cross hatch bar; riluzole 10.0 mg kg^{-1} – shaded bar. Each point represents mean ± s.e. for 10 rats. *$P = 0.05$; †$P = 0.001$ compared with saline controls.

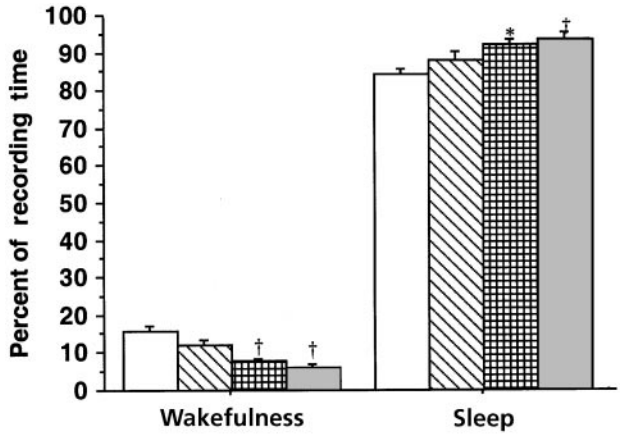

Figure 3 Effect of riluzole (0.5, 5.0 and 10.0 mg kg^{-1}) on wakefulness and total sleep in rats during 6 h polygraphic recordings. Saline control – open bar; riluzole 0.5 mg kg^{-1} – diagonal hatch bar; riluzole 5.0 mg kg^{-1} – cross hatch bar; riluzole 10.0 mg kg^{-1} – shaded bar. Each point represents mean ± s.e. for 10 rats. *$P = 0.002$; †$P = 0.001$ compared with saline controls.

Table 3 Effects of riluzole on sleep/wake architecture in rats.

	% Wakefulness	% NREM	% REM
Control (saline)	15.9 ± 1.9	72.2 ± 2.1	11.9 ± 1.4
Riluzole 0.5 mg kg^{-1}	12.1 ± 2.2	74.3 ± 2.7	13.6 ± 1.9
Riluzole 5.0 mg kg^{-1}	7.7 ± 0.9†	75.8 ± 1.4	16.5 ± 1.1*
Riluzole 10.0 mg kg^{-1}	6.2 ± 1.2†	79.1 ± 1.5*	14.5 ± 1.6

Riluzole (5.0 and 10.0 mg kg^{-1}) decreased wakefulness and increased sleep. All values are mean ± s.e. for percent recording in 10 rats. *$P = 0.04$; †$P = 0.001$ compared with saline controls.

Figure 2 demonstrates the effects of riluzole (0.5, 5.0 and 10.0 mg kg^{-1}) on the rate of spontaneous and post-sigh apnoea expression per hour of REM sleep. There was a significant reduction in post-sigh apnoea index ($P = 0.05$ for 0.5 mg kg^{-1}; $P = 0.05$ for 5.0 mg kg^{-1}; and $P = 0.001$ for 10.0 mg kg^{-1}). As in NREM sleep, spontaneous apnoeas were not affected by the drug at any dose.

Table 3 shows the effects of riluzole on sleep/wake architecture in rats during 6 h of polygraphic recording. Administration of riluzole dose-dependently reduced wakefulness ($P = 0.001$ for 5.0 and 10.0 mg kg^{-1}), and increased NREM ($P = 0.04$ for 10.0 mg kg^{-1}) and REM sleep ($P = 0.04$ for 5.0 mg kg^{-1}). NREM sleep is the major component of sleep and its control value was 72.2 ± 2.1% of the total recording time. Its dose-dependent increase following riluzole administration mirrored the dose-dependent decrease in wakefulness, suggesting that the degree of inhibition of glutamate release could have contributed to the phenomenon.

The effect of riluzole on wakefulness and total sleep is shown in Figure 3 which demonstrates that riluzole dose-dependently reduced wakefulness ($P = 0.001$ for 5.0 and 10.0 mg kg^{-1}) and increased total sleep ($P = 0.002$ for 5.0 mg kg^{-1} and $P = 0.001$ for 10.0 mg kg^{-1}).

Discussion

The major finding of the study was that riluzole dose-dependently suppressed post-sigh apnoeas during REM sleep (Figure 2), a permissive sleep state for both naturally occurring and evoked apnoeas (Carley & Radulovacki 1999a, b). This effect may be attributable to reduced glutamatergic neurotransmission in the CNS (Doble 1996). This finding is of interest because it demonstrates that the mechanisms of apnoea genesis are state dependent and that post-sigh and spontaneous apnoeas arise from at least partially different mechanisms. These conclusions are also supported by our previous report that vagal stimulation produced by peripherally administered serotonin increased the frequency of spontaneous but not post-sigh apnoeas in REM but not in NREM sleep (Carley & Radulovacki 1999a).

The mechanisms underlying apnoeas in rats, as in man, are poorly understood. Thomas et al (1992) demonstrated a differential response of post-sigh and spontaneous apnoeas to several conditioning protocols, and suggested that these two classes of apnoea may be governed by different mechanisms. Riluzole's lack of effect on spontaneous apnoeas could be due to the existence of multiple respiratory modulation regions in the pons with differential effects on respiration resulting from glutamatergic stimulation. In anaesthetized rats, microinjections of glutamate into the lateral parabrachial region produced hyperpnoea (Chamberlin & Saper 1994), while microinjections of glutamate into the ITR, a neighbouring area, caused a reflex apnoea (Chamberlin & Saper 1998). Thus, there are potentially competing regions in the pons for respiration or apnoea expression and each of these regions can be pharmacologically activated by glutamate.

In addition to the existence of potentially competing regions for apnoea expression in the pons, there is also a variety of glutamatergic receptors in the CNS with differential physiological responses to glutamate. In the KF nucleus several glutamatergic and glycinergic antagonists were tested for their potency in blocking reflex apnoea induction: only the *N*-methyl-D-aspartate receptor antagonist AP5 was found to block ethmoidal nerve-evoked respiratory depression (Dutschmann & Herbert 1998). In contrast, the AMPA/kainate receptor antagonist CNQX and the glycine receptor antagonist strychnine were found to be ineffective (Dutschmann & Herbert 1998). The lack of a detailed mapping in the pontine respiratory group according to their distinct physiological roles (Dick et al 1994; Fung et al 1994) contributes to the present difficulty in interpretation of our results. Furthermore, one cannot rule out that alteration of glutamatergic systems outside the pons may have influenced post-sigh apnoea expression.

Our results in Table 1 and Figure 3 show sedative and sleep-inducing effects of riluzole. This is in accordance with the work of Stutzmann et al (1988) who demonstrated that administration of riluzole in doses of 0.5–8.0 mg kg^{-1} to rats reduced wakefulness and increased NREM and REM sleep. In the rat model of absence epilepsy, administration of riluzole to rats produced similar results on sleep (Romettino et al 1991). These general effects of riluzole on sleep/waking are not surprising, since reduced release of glutamate, an excitatory neurotransmitter in the CNS, may be expected to lead to reduced waking and increased sleep.

Our data demonstrate for the first time that glutamate, in addition to its reported role in the mechanism of reflex apnoeas and its action on sleep, plays a role in the genesis of the post-sigh apnoeas during REM sleep.

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