Respiratory sinus arrhythmia in freely moving and anesthetized rats

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Bouairi, Evgenia, Robert Neff, Cory Evans, Allison Gold, Michael C. Andresen, and David Mendelowitz. Respiratory sinus arrhythmia in freely moving and anesthetized rats. J Appl Physiol 97: 1431–1436, 2004.—Heart rate increases during inspiration and slows during postinspiration; this respiratory sinus arrhythmia helps match pulmonary blood flow to lung inflation and maintain an appropriate diffusion gradient of oxygen in the lungs. This cardiorespiratory pattern is found in neonatal and adult humans, baboons, dogs, rabbits, and seals. Respiratory sinus arrhythmia occurs mainly due to inhibition of cardioinhibitory parasympathetic cardiac vagal neurons during inspiration. Surprisingly, however, a recent study in anesthetized rats paradoxically found an enhancement of cardiac vagal activity during inspiration, suggesting that rats have an inverted respiratory sinus arrhythmia (Rentero N, Cividjian A, Trevaks D, Pequignot JM, Quintin L, and McAllen RM. Am J Physiol Regul Integr Comp Physiol 283: R1327–R1334, 2002). To address this controversy, this study examined respiratory sinus arrhythmia in conscious freely moving rats and tested whether the commonly used experimental anesthetics urethane, pentobarbital sodium, or ketamine-xylazine alter respiratory sinus arrhythmia. Heart rate significantly increased 21 beats/min during inspiration in conscious rats, a pattern similar to the respiratory sinus arrhythmia that occurs in other species. However, anesthetics altered normal respiratory sinus arrhythmia. Ketamine-xylazine (87 mg/kg and 13 mg/kg) depressed and pentobarbital sodium (60 mg/kg) abolished normal respiratory sinus arrhythmia. Urethane (1 g/kg) inverted the cardiorespiratory pattern so that heart rate significantly decreased during inspiration. Our study demonstrates that heart rate normally increases during inspiration in conscious, freely moving rats, similar to the respiratory sinus arrhythmia pattern that occurs in other species but that this pattern is disrupted in the presence of general anesthetics, including invernessation in the case of urethane. The presence and consequences of anesthetics need to be considered in studying the parasympathetic control of heart rate.

during each respiratory cycle, the heart beats more rapidly in inspiration and slows during postinspiration, a pattern that is referred to as respiratory sinus arrhythmia. Such increases in heart rate during inspiration are observed in a wide variety of mammals, including both neonatal (17) and adult humans (10, 19), baboons (34), dogs (18), rabbits (26), seals (6), and squirrels (16). Respiratory sinus arrhythmia benefits pulmonary gas exchange by improving ventilation-to-perfusion ratios within each respiratory cycle (18, 29, 46). Diminished respiratory sinus arrhythmia occurs in many disease states. In distressed fetuses, as well as partially asphyxiated newborns, respiratory sinus arrhythmia is reduced, and this is well correlated (independent from heart or respiratory rate) with low Apgar scores and neonatal mortality in newborns (9, 32, 39). Because heart rate variability is caused mainly by respiratory sinus arrhythmia, the mechanisms responsible for this pattern may be adversely affected in association with many cardiovascular diseases, including diabetic autonomic neuropathy, hypertension, myocardial infarction, and heart failure, in which respiratory sinus arrhythmia is abnormally low (44).

Although feedback from pulmonary stretch receptors and direct respiratory-related changes in venous return and cardiac stretch can evoke respiratory-related fluctuations in heart rate, the dominant source of respiratory sinus arrhythmia originates from the brain stem (1). Respiratory sinus arrhythmia persists when the lungs are stationary (caused by muscle paralysis or constant flow ventilation), and the respiratory modulation of heart rate remains synchronized with brain stem respiratory rhythms even if artificial ventilation of the lungs, and chemoreceptor activation, occurs at different intervals (7, 13, 20, 41, 43). Results in subjects hyperventilated with 100% oxygen indicate respiratory modulation of heart rate occurs via central cardiorespiratory interactions rather than respiratory movement or lung inflation (11).

The respiratory system also influences heart rate by modulating the baroreceptor and chemoreceptor input to cardiac vagal neurons. In both animals and humans, the baroreceptor and chemoreceptor reflexes are inhibited during inspiration, and they are facilitated during postinspiration and expiration or during a maintained phase of postinspiration and apnea (8, 11, 12, 30). Cardiac vagal responses in humans are greater when the baroreceptors are stimulated by brief neck suction applied in expiration than inspiration (10). This respiratory modulation of both reflexes persists after pulmonary denervation, as well as ventilatory paralysis, suggesting that this “gating” of the baroreceptor and chemoreceptor reflexes also occurs within the brain stem (29).

In animals, including humans, respiratory sinus arrhythmia is mediated almost exclusively via changes in parasympathetic cardiac vagal activity. Respiratory sinus arrhythmia persists in experimental animals on disruption of sympathetic pathways and in quadriplegic patients with spinal cord injury and sympathetic dysfunction (7, 13, 20, 21, 41). Pharmacological blockade of parasympathetic cardiac activity in dogs abolishes respiratory sinus arrhythmia, whereas blocking sympathetic cardiac activity with propranolol has little effect (49). Similar
abolishment of the normal pattern of respiratory sinus arrhythmia in the squirrel is observed with application of the parasympathetic blocker atropine (16).

Cardioinhibitory parasympathetic cardiac activity follows a pronounced respiratory pattern. Cardiac vagal fibers (recorded from fibers in the cardiac nerve in the cat) fire most rapidly in postinspiration and are often silent in inspiration (27). Similarly, cardiac vagal neurons recorded in the brain stem nucleus ambiguus (also in the cat) are inhibited during inspiration and most active during postinspiration (14, 42). Thus cardioinhibitory cardiac vagal neurons are inhibited during inspiration and/or are excited during postinspiration. However, a recent study in rats paradoxically reported that the activity of cardiac vagal neurons was enhanced in vivo during inspiration, leading to the suggestion that rats have an inverted respiratory sinus arrhythmia (37). In contrast, in the rat working heart brain stem preparation, heart rate increases during inspiration in a pattern consistent with observations in other mammals (36).

Several factors might contribute to the conflicting results observed in these different studies that used rats. The presence and choice of general anesthetic might well critically alter parasympathetic cardiac activity (31). This study tests the hypotheses that conscious rats normally display a pattern of respiratory sinus arrhythmia comparable to other mammals and that respiratory sinus arrhythmia may be altered by commonly used experimental anesthetics such as urethane, pentobarbital sodium, and ketamine-xylazine. Our results demonstrate that conscious rats exhibit the common mammalian pattern of inspiratory acceleration of heart rate and that, whereas most general anesthetics depress this pattern, urethane inverts the pattern of normal respiratory sinus arrhythmia in the rat.

MATERIALS AND METHODS

Adult female Sprague-Dawley rats were anesthetized with a combination of ketamine (87 mg/kg) and xylazine (13 mg/kg ip; Phoenix Pharmaceuticals, St. Joseph, MO). The left femoral artery was exposed and catheterized with sterile Micro-Renthane tubing (Braintree Scientific, Braintree, MA) that had been soaked overnight in heparinized bacteriostatic saline. Animals were placed in a Covance infusion harness (Instech Laboratories, Plymouth Meeting, PA) and allowed to recover for 48 h. After recovery, animals were placed in a whole body plethysmograph chamber, which allowed simultaneous measurement of blood pressure, heartbeat intervals, and respiratory airflow in unanesthetized, freely moving rats using Biosystem XA software (Buxco Electronics, Sharon, CT). After a 5-min period of recording while the animal was awake and sedentary, the animal was then anesthetized with either ketamine-xylazine (87 mg/kg and 13 mg/kg), pentobarbital sodium (60 mg/kg), or urethane (1 g/kg). Measurements of blood pressure, heartbeat intervals, and respiratory activity were then repeated while the animal was anesthetized, after which the animal was killed by an overdose of pentobarbital sodium (100 mg/kg). Twenty-three rats were used in this study, and a different group of animals (ketamine-xylazine n = 8, pentobarbital sodium n = 8, and urethane n = 7) was used to examine the effects of each anesthetic. All animal procedures were approved by George Washington University’s Institutional Animal Care and Use Committee, and they were performed in accordance with the recommendations of the panel on euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication Guide for the Care and Use of Laboratory Animals.

Heartbeat intervals were measured during inspiratory and subsequent expiratory periods by using Acknowledge v3.7.3 (Biopac Systems, Goleta, CA). Inspiration was defined as the period from the onset of inspiratory airflow to the onset of expiratory airflow. Inspiration and expiratory heartbeat intervals were taken as the first full heartbeat interval during the period of inspiration and the last full heartbeat interval during expiration, respectively. The data from at least 20 respiratory cycles of inspiratory and expiratory heartbeat intervals were averaged for each animal while the animal was awake and sedentary and then at least 20 respiratory cycles during anesthesia. Data are presented as averages ± SE. Statistical comparisons were made by using paired Student’s t-tests.

RESULTS

Respiratory activity and arterial blood pressure were simultaneously recorded in chronically instrumented conscious and sedentary rats as shown in Fig. 1A. As shown in this typical experiment, the heartbeat interval decreased with each inspiration. The individual and average results from eight rats are shown in Fig. 1B. The average interbeat interval significantly increased from 0.153 ± 0.005 s during inspiration to 0.162 ± 0.005 s during expiration. This change in interbeat interval corresponds to an inspiratory-related increase in heart rate of 21 beats/min.

After the animals were anesthetized with ketamine-xylazine (87 mg/kg and 13 mg/kg), the heartbeat interval significantly increased from 0.153 ± 0.005 to 0.214 ± 0.006 s; this corresponds to a decrease in heart rate from 395 ± 16 to 281 ± 7 beats/min. Blood pressure also significantly increased from 124 ± 4 to 163 ± 6 mmHg with ketamine-xylazine, and the frequency of respiration significantly decreased from 3.4 ± 0.1 to 2.5 ± 0.2 Hz. Ketamine-xylazine did not significantly alter tidal volume, inspiratory time, or expiratory time (control 124 ± 22, ketamine-xylazine 72 ± 7 ml; control 0.17 ± 0.01, ketamine-xylazine 0.20 ± 0.01 s; control 0.48 ± 0.04, ketamine-xylazine 0.71 ± 0.09 s, respectively). Respiratory sinus arrhythmia persisted while the animals were anesthetized with ketamine-xylazine (87 mg/kg and 13 mg/kg) because the heartbeat interval significantly decreased from 0.214 ± 0.006 s during inspiration to 0.221 ± 0.006 s during expiration. This change in heart period corresponds to an inspiratory-related increase in heart rate of 8 beats/min.

Unlike ketamine-xylazine, pentobarbital sodium did not significantly alter heart rate. Pentobarbital sodium did significantly decrease blood pressure from 113 ± 6 to 89 ± 7 mmHg, and the frequency of respiration significantly decreased from 3.5 ± 0.1 to 2.4 ± 0.2 Hz. Pentobarbital sodium did not significantly alter tidal volume or expiratory time, but it did increase inspiratory time from 0.19 ± 0.01 to 0.23 ± 0.01 s. Also, in contrast to ketamine-xylazine, which blunted respiratory sinus arrhythmia, pentobarbital sodium (60 mg/kg) abolished respiratory sinus arrhythmia. As shown in Fig. 2, before the anesthetic, the heartbeat interval increased in each of the eight conscious rats during expiration. The average interbeat interval significantly increased from 0.151 ± 0.005 s during inspiration to 0.158 ± 0.004 s during expiration. This change in heart period corresponds to an inspiratory-related increase in heart rate of 19 beats/min. After the animals were anesthetized with pentobarbital sodium (60 mg/kg), the heartbeat interval did not change significantly from the intervals before anesthesia. During inspiration, the heart rate interval was not significantly different from the heartbeat interval during expiration (0.159 ± 0.006 s and 0.161 ± 0.005 s for inspiration and expiration, respectively; P > 0.2).

Urethane (1 g/kg) did not significantly alter blood pressure, respiratory frequency, or inspiratory time, but it did signifi-
significantly increase tidal volume and decrease expiratory time (from 99 ± 10 to 120 ± 5 ml and from 0.49 ± 0.05 to 0.29 ± 0.02 s, respectively). Surprisingly, urethane inverted normal respiratory sinus arrhythmia. As shown from a typical experiment in Fig. 3A and in the summary data from seven rats (Fig. 3B), before the application of urethane, conscious animals had a normal respiratory sinus arrhythmia in which heartbeat interval consistently decreased during expiration. The average interbeat interval significantly increased from 0.153 ± 0.005 s during inspiration to 0.162 ± 0.005 s during expiration. After the animals were anesthetized with ketamine-xylazine (87 mg/kg and 13 mg/kg), the heartbeat interval significantly increased from 0.153 ± 0.005 to 0.214 ± 0.006 s (right). Respiratory sinus arrhythmia persisted while the animals were anesthetized with ketamine-xylazine (87 mg/kg and 13 mg/kg) because the heartbeat interval significantly increased from 0.214 ± 0.006 s during inspiration to 0.221 ± 0.006 s during expiration. *P ≤ 0.05; **P ≤ 0.01.

Fig. 1. A: respiratory activity and arterial blood pressure were simultaneously recorded, and heartbeat interval was calculated, in chronically instrumented conscious and sedentary rats. Heartbeat interval consistently decreased with each inspiration. B: inspiratory and expiratory heartbeat intervals from each of the 8 rats (dashed lines) and the average (±SE) interbeat interval (solid line). Average interbeat interval significantly increased from 0.153 ± 0.005 s during inspiration to 0.162 ± 0.005 s during expiration. After the animals were anesthetized with ketamine-xylazine (87 mg/kg and 13 mg/kg), the heartbeat interval significantly increased from 0.153 ± 0.005 s to 0.214 ± 0.006 s (*right). Respiratory sinus arrhythmia persisted while the animals were anesthetized with ketamine-xylazine (87 mg/kg and 13 mg/kg) because the heartbeat interval significantly increased from 0.214 ± 0.006 s during inspiration to 0.221 ± 0.006 s during expiration. *P ≤ 0.05; **P ≤ 0.01.

Fig. 2. In a second group of animals, before the anesthetic, the heartbeat interval increased in each of the 8 freely moving rats during expiration. Inspiratory and expiratory heartbeat intervals from each of the 8 rats (dashed lines) and the average intervals (±SE; solid line) are shown. Average interbeat interval significantly increased from 0.151 ± 0.005 s during inspiration to 0.158 ± 0.004 s during expiration. After these animals were anesthetized with pentobarbital sodium (60 mg/kg), the average heart rate interval during inspiration was not significantly different from the average heartbeat interval during expiration (0.159 ± 0.006 s and 0.161 ± 0.005 s for inspiration and expiration, respectively; *P > 0.2). *P ≤ 0.05; **P ≤ 0.01.

**DISCUSSION**

There are two major results from this study: 1) heart rate increases during inspiration and decreases during expiration in conscious, freely moving rats, and 2) anesthetics alter normal respiratory sinus arrhythmia. Whereas ketamine-xylazine depressed normal respiratory sinus arrhythmia, pentobarbital so-
that the anesthetic urethane alters cardiorespiratory interactions conflicting results observed by others. Our results demonstrate general reduce or eliminate parasympathetic cardiac activity. However, there are several factors that make the latter study in rats and that heart rate would decrease during inspiration. This would predict that respiratory sinus arrhythmia is inverted paradoxically shown that the activity of cardioinhibitory cardboard abolished respiratory sinus arrhythmia. However, urethane paradoxically inverted the pattern of respiratory sinus arrhythmia such that heart rate decreased during inspiration. Data describing cardiorespiratory interactions in the rat have been controversial. In the rat working heart-brain stem preparation (a preparation without anesthetics), heart rate increases during inspiration (36). However, a more recent study has paradoxically shown that the activity of cardioinhibitory cardiac vagal neurons is enhanced during inspiration in rats (37). This would predict that respiratory sinus arrhythmia is inverted in rats and that heart rate would decrease during inspiration. However, there are several factors that make the latter study difficult to interpret, including the use of anesthetics that in general reduce or eliminate parasympathetic cardiac activity. The work presented here provides a likely explanation for the conflicting results observed by others. Our results demonstrate that the anesthetic urethane alters cardiorespiratory interactions in the rat and produces an inversion of the normal phasing of this cardiorespiratory pattern. This action of urethane is likely responsible for the recent observation of paradoxical enhancement of the activity of cardiac vagal neurons during inspiration in rats (37). Our results demonstrate that, normally, heart rate increases during inspiration in conscious, freely moving rats, similar to the pattern of respiratory sinus arrhythmia commonly reported for mammals including humans.

The cellular mechanisms likely responsible for respiratory sinus arrhythmia have recently been examined. In an in vitro study, rhythmic inspiratory-related activity was recorded from the hypoglossal rootlet while synaptic events were recorded in identified cardiac vagal neurons in the nucleus ambiguus (35). During inspiratory bursts, the frequencies of both spontaneous inhibitory GABAergic and spontaneous glycinergic synaptic events in cardiac vagal neurons were significantly increased. Focal application of the nicotinic antagonist dihydro-β-erythroidine to an area immediately surrounding the patched cardiac vagal neuron, in an α4β2-selective concentration (3 μM), abolished the respiratory-evoked increase in GABAergic frequency. In contrast, the increase in glycinergic frequency during inspiration was not altered by nicotinic antagonists. Interestingly, prenatal nicotine exposure exaggerated the increase in GABAergic frequency during inspiration and enhanced GABAergic synaptic amplitudes both between and during inspiratory events, but glycinergic synaptic frequency and amplitude were unchanged by prenatal nicotine exposure.

Although the mechanisms by which general anesthetics alter respiratory sinus arrhythmia are poorly understood, brain stem mechanisms are clearly important (28). Anesthetics appear to have multiple sites of action on cardiac vagal neurons (22–24). Ketamine is associated with a hemodynamic profile that typically is characterized by increases in both blood pressure and a depression of the baroreflex control of heart rate, as shown in awake conscious dogs (47, 48) and rabbits (4, 5). Recent work has shown that ketamine, at clinically relevant concentrations, inhibits the magnitude and enhances the inactivation of voltage-gated sodium currents in cardiac parasympathetic neurons (23). Ketamine did not alter the voltage-gated potassium currents (23). Ketamine also inhibits excitatory synaptic inputs to cardiac parasympathetic neurons. Ketamine inhibits both presynaptic nicotinic cholinergic receptors, which play a facilitory role in excitatory glutamatergic neurotransmission, and also the responses of postsynaptic nicotinic receptors, which act to directly depolarize cardiac parasympathetic neurons (24).

Barbiturates, such as pentobarbital, can cause central respiratory depression and blunting of cardiovascular homeostatic reflexes (2, 3, 33, 45, 50). Results from animal studies have shown that pentobarbital decreases the gain of the baroreceptor reflex on the order of 50%, and this blunting of the baroreflex is usually nearly entirely by decreasing cardioinhibitory parasympathetic activity that dominates the control of heart rate (3, 45). Pentobarbital, at clinically relevant concentrations, alters the activity of cardiac vagal neurons by prolonging the duration of spontaneous GABAergic inhibitory postsynaptic currents that impinge on cardiac parasympathetic neurons. Expression of the GABAₐ receptor ϵ-subunit in cardiac parasympathetic neurons renders the GABA receptor insensitive to pentobarbital (22).

Urethane has been shown to weaken the baroreflex control of heart rate, as well as diminish the responses to carotid occlusion, tilt, and sodium cyanide (25, 38). However, the mechanisms of action of urethane are poorly understood.

Fig. 3. In a third group of animals, before the application of urethane, the average heartbeat interval significantly increased during expiration. A: results from a typical experiment. B: combined results from 7 animals. Results from each of the 7 animals (dashed lines) and average results (±SE; solid line) are shown. Average interbeat interval significantly increased from 0.176 ± 0.004 s during inspiration to 0.183 ± 0.005 s during expiration. After the animals were anesthetized with urethane (1 g/kg), the respiratory-related changes in heartbeat interval significantly decreased from 0.170 ± 0.005 s during inspiration to 0.166 ± 0.005 s during expiration. *P < 0.05.
Urethane decreases paired-pulse inhibition in the dentate gyrus and reduces evoked and spontaneous firing in the rat sensorimotor striatum (40, 51). Urethane evokes modest and broad changes in receptor function, potentiating the function of nicotinic, GABA<sub>A</sub> and glycine receptors, while inhibiting glutamatergic AMPA and NMDA receptor responses (15). The mechanisms responsible for urethane inverting respiratory sinus arrhythmia, as shown in this study, are unknown. It would be interesting to examine the mechanisms underlying this effect of urethane on the activity of cardiac vagal neurons, especially in comparison to the known effects of ketamine and pentobarbital, which alter synaptic neurotransmission and postsynaptic voltage-gated currents in cardiac vagal neurons (22–24).

In summary, this study demonstrates that conscious and freely moving rats have a normal pattern of respiratory sinus arrhythmia in which heart rate increases during inspiration and decreases during expiration, which is common to other mammals. However, the anesthetic urethane disturbs these cardiorespiratory interactions and inverts respiratory sinus arrhythmia to an abnormal pattern of activity. Thus the actions of general anesthetics need to be considered in studies of the activity of cardiac vagal neurons as well as the parasympathetic control of heart rate, and whenever possible the use of anesthetics should be avoided.

REFERENCES

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