Repetitive episodic hypoxia every 30 sec administered chronically to Sprague-Dawley (SD) rats has been shown by previous studies to cause a sustained increase in daytime blood pressure (BP). Acoustic arousal in humans during wake or sleep produces an acute BP rise. The question then arises as to whether chronic episodic acoustic arousal applied with the same frequency and duration as episodic hypoxia induces elevated BP. We exposed 14-week-old (N = 10) SD rats in individual cages to recurrent buzzer noise (500 Hz, 100 dB) 6 out of every 30 sec, 7 h/day for 35 days. Ten other rats were placed in similar cages daily but not exposed to noise, to provide a sham condition. An infrared beam with a detector was positioned at the end of each cage. This allowed us to quantify motion by registering the number of times the rat broke the beam per 7 h period. Mean intraarterial BP was measured in unrestrained conscious animals at baseline and at the end of 35 days of their respective conditions. Acute episodic acoustic stimulation caused an immediate response in BP and heart rate. Habituation occurred in that the movement response to 120 noises per hour was 75% in hour one and 20% in hours two through seven on day one. The movement response was further reduced by day 35 but remained significantly higher than in animals not stimulated by noise. The cardiovascular response to noise also showed signs of habituation. Chronic noise stimulation produced no sustained increases in BP after 35 days of exposure. Am J Hypertens 1999; 12:504–510

KEY WORDS: Stress, blood pressure, sympathetic nervous system, blood pressure, sleep disorders, sleep apnea, hemodynamics, arousal, noise, Sprague-Dawley rat.

Accumulating evidence from epidemiologic studies associates obstructive sleep apnea (OSA) with sustained daytime (diurnal) hypertension. The mechanism for chronic increase in blood pressure (BP) may be multifactorial. Episodic hypoxemia, accentuated intrathoracic pressure changes with shifts in blood volume, recurrent arousals, and sleep architecture disruption are considered potential BP elevating stimuli. The degree of hypoxia during repetitive obstructive apneas is directly related to the degree of acute BP change and supplemental oxygen administered to humans simulating obstructive apnea minimizes the degree of acute BP change. On the other hand, arousal from sleep has been shown to cause an acute increase in BP in humans and is believed by some to be important in the acute BP change of OSA. The magnitude of BP increase during sleep has been shown to be proportionate to the grade of acoustic arousal in humans. One study however, shows no increase in BP in response to chronic recurrent acoustic arousal in dogs.
In a previously described rat model, a rapid flux of nitrogen into individual Plexiglas chambers lowers ambient oxygen to create episodic (every 30 sec) hypoxia, simulating the cyclic desaturation seen in humans with OSA. We have demonstrated a consistent, sustained elevation in diurnal BP in rats following 35 days of such treatment. Based upon informal observations of behavior in previous studies, animals in the episodic hypoxia chambers appear stressed. While maintaining the same diurnal sleep cycle, the hypoxia rats sleep fitfully, often exhibit behavioral arousal during nadir hypoxic periods, and turn and reposition themselves frequently. At the same time, those in sham cages (compressed air infusion at a frequency equal to episodic hypoxia) appear oblivious to noise or exchanges of air. Thus, it is possible that stress related to sleep disruption and arousal in the episodic hypoxia rats contributed to the persistent increase in BP. Acoustic stimulation in many forms has been shown to induce both acute and chronic cardiovascular responses in humans as well as animals. Chronic stress and increased sympathetic nerve activity is postulated to contribute to primary hypertension. In the current study, we designed an acoustic system that mimicked the timing, pattern, and duration of the episodic hypoxia and compressed air stress, but with a much greater noise intensity to insure recurrent movement-arousal. We hypothesized that acoustic arousal would not induce a chronic BP response as does episodic hypoxia. We took this position because we believe that it is the episodic hypoxia that creates the restlessness and motion disturbance in our model, rather than the low level of noise in our system.

**METHODS**

Twenty 14-week old, 350 to 400 g, male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used for the study. The animals were divided into two groups and housed in identical cylindrical Plexiglas chambers (length 28 cm, diameter 10 cm, volume 2.4 L) from approximately 09:00 to 16:00 each day of the experimental period. The chambers provided sufficient room for the rats to move freely lengthwise and to turn. In the study group, a buzzer that produced a noise of 500 Hz and 100 dB (Guardian Electric Manufacturing Co., Woodstock, IL) was attached to the bottom of each chamber. Using an electric time switch (Dayton Electric Co., Chicago, IL), the noise was applied for 6 sec, twice each minute. During the experiment, the animals in the study group were exposed to episodic noise for 7 h daily for 35 days, while the controls in similar cages remained in a quiet environment. An infrared photoelectric sensor (Radio Shack, Forth Worth, TX) was directed through the chamber toward the head of the animal. Whenever the animal crossed the beam, the interruption was recorded.

At least 24 h before measurement of BP and heart rate (HR) the animals were anesthetized with a cocktail consisting of ketamine, acepromazine, and xylazine and an arterial catheter (Silastic, internal diameter 0.03 mm, in Tygon, internal diameter 0.05 mm) was inserted via the right femoral artery into the abdominal aorta. The catheter was exteriorized at the nape of the neck for recording BP and HR in conscious unrestrained animals. The catheters were maintained patent during the experimental period by flushing with a heparin solution. Each rat required BP measurements 2 days before and 2 days after the 35 day period. Twenty-four hours before experimental day 1 and within 48 h following experimental day 35, under resting, unrestrained conditions, the catheters were attached to Statham P23Db pressure transducers with signal amplification (Hewlett Packard Co., 7858B, Andover, MA) to measure baseline and follow-up BP, respectively. On these days, HR and mean arterial pressure (MAP) were measured over 3 to 4 h, between 09:00 and 13:00. The lowest stable MAP recorded continuously for 10 min or more was taken as the value for the recording session. Also, within 48 h before beginning and within 48 h following the 35-day experimental period, both groups of animals were challenged with episodic acoustic stimulation for 7 h and the acute BP and HR responses recorded continuously. BP and HR values from five cycles at 5 min and at 30 min were recorded, subtracting baseline from peak values for each cycle. The mean of five data points was used as a single data point for each rat. Means for each group of 10 rats were then compared statistically.

Data are expressed as mean with standard error of the mean (SEM). Data were compared by paired t test for within-group comparisons (eg, baseline to follow-up values), and by unpaired t test for between-group comparisons. Means for two or more conditions were compared using analysis of variance (ANOVA) followed by Bonferroni’s test and Student’s t test as post hoc tests when applicable. Statistical significance was accepted when \( P < .05 \).

**RESULTS**

Episodic acoustic stimulation aroused the study animals as evidenced by significantly more movements during the 7 h diurnal (during behavioral sleep) exposure when compared to controls not exposed to noise (52.9 vs 33.8 movements/h respectively for the first hour, and 30.8 vs 25.3 movements/h respectively for the seventh hour) (Figure 1). The daily measured movements show that the noise-exposed animals had significantly higher movement over the 5-week study period (Figure 2). The largest daily movement differ-
ence between the study and control groups was on day 29 (249.2 v 125.8 movements) and smallest was on day 23 (154.7 v 139.2 movements) (Figure 2).

In contrast to previous studies showing a 10 to 14 mm Hg increase in MAP when exposed to episodic hypoxia, the resting MAP before and after the 5-week noise exposure in this study was not different (Figure 3). The resting, unstimulated HR, however, was about 10% lower at the end of 35-day exposure in both noise-exposed and control groups, as observed in previous studies.8,9,14

Episodic acoustic stimulation evoked acute, periodic elevation of BP with a biphasic HR (bradycardia-tachycardia), which attenuated within 30 min of repetitive exposure. At baseline, the change in MAP with acute acoustic challenge in the study and control groups during the first 5 min of exposure was 24.6 ± 2.9 mm Hg (HR = 33 ± 4 beats/min) and 19.7 ± 1.6 mm Hg (HR = 30 ± 3 beats/min) respectively (P = NS) (Figure 4). After 5 weeks of exposure to episodic noise, the MAP change with acute acoustic challenge in the first 5 min exposure was 8.4 ± 1.4 mm Hg (HR = 9 ± 2 beats/min) in the study group compared to 19.2 ± 1.0 mm Hg (HR = 18 ± 4 beats/min) in controls (P < .05). At baseline, the change in MAP during acute acoustic challenge after 30 min exposure

FIGURE 1. Movements per hour recorded hourly over a 7-h exposure to repetitive noise stimulation of 6 sec duration, every 30 sec in 10 acoustically stimulated rats (solid lines and dots) versus 10 unstimulated (broken lines and open circles) control rats housed in identical chambers. Top: data for day 1 only. Bottom: data for day 35 only. Although the movement response after the first hour is attenuated, especially on day 35, movements per hour for study rats remain significantly (P < .05) higher at all hours on both days.

FIGURE 2. Number of movements per day over a 35-day period in acoustically stimulated rats (solid line and dots) versus nonaroused control rats (broken lines and open circles). Differences between means of stimulated and control rats are significant on all days P < .01. In this and subsequent figures, error bars are ± one standard error.

FIGURE 3. Mean arterial pressure (MAP) and heart rate (HR) at baseline and at end of a 35-day period for both chronic acoustic arousal rats (solid line and closed circles) and nonaroused rats (broken line and open circles). All measurements were made in conscious, unrestrained, nonsedated, nonstimulated rats. There is no effect of chronic, daily acoustic stimulation on BP as evidence by lack of significant change over the 35-day period either in the experimental or control group. Both groups showed a significant drop in HR from baseline (*P < .05) at 35 days, which may reflect aging or acclimation to handling, or both.
in the study and control groups was $15.7 \pm 3.3\ mm\ Hg$ (HR = $18 \pm 3\ beats/min$) and $15.7 \pm 2.0\ mm\ Hg$ (HR = $29 \pm 5\ beats/min$) respectively ($P = NS$) (Figure 5). After 5 weeks of exposure to episodic noise, the MAP change with acute challenge after 30 min exposure in the study and control groups was $11.8 \pm 0.8\ mm\ Hg$ (HR = $10 \pm 2\ beats/min$) and $14.8 \pm 1.6\ mm\ Hg$ (HR = $17 \pm 4\ beats/min$), respectively ($P = NS$). There was no difference between the 5 min and 30 min BP response within the study (8.4 v 11.6 mm Hg) or control (19.2 v 14.8 mm Hg) groups after 35 days. Thus, the response to acute acoustic stimulation at 5 min remained intact in the controls not exposed to noise over 35 days while the chronic, acoustically stimulated rats decreased their acute (first 5 min) BP response. The HR change was also significantly lower after 35 days of exposure in the study group. In contrast, at 30 min of episodic noise, BP and HR responses were lower than the 5 min values and remained low for both groups after 35 days (Figure 5). This is probably due to the fact that the response to 30-min stimulation is greatly attenuated when compared to 5-min responses. Therefore, attenuation is minimal because the response is minimal at outset.

**DISCUSSION**

Although hypoxemia and arousal have been shown to acutely elevate blood pressure in both humans and experimental animals, no evidence exists that repetitive arousal alone causes sustained elevation of diurnal BP. The goal of this study was to determine if repetitive arousal for approximately 7 h/day for 35 days contributed to the diurnal BP elevation previously demonstrated in this model. The reason for the study was to try to tease out contributing factors in this chronic, diurnal BP elevation model. Episodic hypoxia may be considered both chemical (hypoxia) as well as neurophysiologic (noise-arousal) stress, either of which could affect central nervous system homeostasis, acute and chronic sympathetic output, and vasomotor tone. The main findings of this study are that: 1) acute episodic acoustic stimulation caused an immediate, vasomotor response in BP and HR, 2) the rat rapidly (daily) habituated in both behavioral (plateaued within 120 min) and cardiovascular response (BP change unmeasurable after 30 min.) with acute noise stimulation. Chronic 35-day noise exposure caused 3) habituation of the 5-min cardiovascular response but not the 30-min response (as it was already low) and 4) chronic noise stimulation produced no sustained increases in BP after 35 days of exposure.

The stress response evolved to allow a rapid reaction to perceived or real threats to survival or well-being of the organism, preserving the internal milieu by short-term adjustments in activities of homeostatic mechanisms.13,14 Such adjustments include changes in heart rate, BP, cardiac output, vasomotor tone, glycolysis, and gluconeogenesis. Stresses that may invoke these responses include psychologic (fear, anger, classical conflict paradigm), physical (tactile, pain, noise), pharmacologic (drugs, hypoxia, hypoglycemia), and hypovolemic (hemorrhage, sepsis, etc) stresses, among others. These stresses acutely invoke sympathetic responses involving the cardiovascular centers of the ventrolateral medulla, with hypothalamic modification mediated by spinal efferents, and activating...
the peripheral sympathetic nervous system, the adrenal medulla, and various hormones. In a series of papers, Folkow has presented data in rats and postulated that frequent repetition of conditioned defense responses in genetically susceptible individuals can lead to structural cardiovascular adaptations, escalating BP to higher levels.\textsuperscript{10,11} Despite many studies, there is no direct evidence that in otherwise healthy humans, repeated, inappropriate or exaggerated stress causes chronic cardiovascular disease that persists after removal of the stressor.\textsuperscript{12}

The analogy of this rat model of episodic hypoxia to actual pathophysiologic events in OSA in humans must be placed in perspective. This model accurately reproduces the oxyhemoglobin desaturation seen in repetitive obstructive apneas in humans and animals. It does not, however, duplicate episodic negative intrathoracic pressure changes, breath holding, fluctuations in cardiac output or intrathoracic blood volume, or the eucapnia-hypercapnia blood gas changes that result from obstructive breathing. Thus, we are examining only one aspect of the pathophysiologic consequences of OSA. It is not clear which of these variables may or may not be important in the development of BP change. Two examples are intrathoracic pressure variation and apneic CO\textsubscript{2} fluctuation. Obstructed apneas in spontaneously breathing baboons versus non-obstructed apneas (using neuromuscular blockade with mechanical ventilation) of the same duration and desaturation levels cause identical acute systemic BP changes during and immediately post-apnea.\textsuperscript{15} Wide intrathoracic pressure variation accompanying obstruction has no acute effect on BP. Other work by O’Donnell et al using blockade of the autonomic nervous system in dogs with obstructive apnea shows an absence of mechanical effects of obstructive apnea.\textsuperscript{16} Likewise, intraapneic CO\textsubscript{2} change may have little effect upon chronic BP change. In male Sprague-Dawley rats, CO\textsubscript{2} added to the hypoxia circuit to create intermittent, episodic eucarbic, or hypercarbic hypoxia has no additional effect upon the MAP increase after 35 days compared to hypocapnic hypoxia.\textsuperscript{14}

Because hypoxia and arousal resulting from apnea are difficult to separate in the natural setting, evidence that hypoxia is the main trigger of acute BP elevation in OSA is indirect. Examining repetitive obstructive apneas in 10 subjects with simultaneous intraarterial BP measurement, Shepard found a direct correlation between the magnitude of each oxyhemoglobin desaturation and the corresponding acute elevation of BP \( R = 0.58 \).\textsuperscript{5} Van Den Aardweg et al had seven awake males simulate apnea with a voluntary breath-hold under hypoxic conditions (allowing desaturation) and during pretreatment with 100% oxygen (precluding desaturation).\textsuperscript{3} Intraapneic BP rose during the hypoxic series but remained stable during hyperoxic apneas, indicating that hypoxia was a necessary component for intraapneic BP rise. Since the subjects were already awake, arousal as a mechanism was not examined. Measuring arterial BP during an hypoxic ramp test, Hedner et al found that OSA patients have a distinct pressor response to hypoxia not present in nonapneic controls.\textsuperscript{17}

Other studies have found that hypoxia may not be the major mechanism inducing acute BP elevation in the setting of sleep apnea. Ringler et al examined 11 OSA patients during sleep, finding that apneas recorded during oxygen supplementation (without desaturation) were associated with equivalent postapneic MAP elevations compared to apneas without oxygen supplementation (with desaturation).\textsuperscript{4} Furthermore, when hypoxemia to arterial saturations of 80% was induced with nitrogen, elevation of BP was not seen if respiratory and sleep disruption were avoided. Finally, in five sleeping subjects, auditory arousal alone caused acute MAP elevation. A subsequent study by the same authors had subjects simulate timed obstructive apneas, comparing BP changes while awake to those during apneas with similar duration and negative intrathoracic pressure during sleep.\textsuperscript{5} Apneas during sleep (accompanied by arousals) produced higher acute BP changes than awake, simulated apneas. By varying stimulus length, transient acoustic stimuli were administered to five sleeping humans creating a range of graded cortical electroencephalograph (EEG) arousals.\textsuperscript{6} During non-rapid eye movement (REM) sleep, there was a trend toward larger BP rises with higher grades of arousal and the average BP rise in response to grade 2 arousal was about 75% of that produced by obstructive apnea. Thus, it appears the immediate BP response to acute apnea could be a combination of arousal and hypoxemia. The role of arousal in the chronic diurnal BP response to recurrent nocturnal apneas is unclear.

The methodology for directly measuring arousals requires implanted skull electrodes with direct physical connection to an EEG, which because of the tether, limits movement of the animal. Remote EEG telemetry devices, while available for larger animals, such as dogs, are too large for rodents. Thus, we chose movement as an indirect indicator of arousal since, presumably, the animal must be aroused from sleep in order to move. A previous study in nine adult rats using noise conditions very similar to ours, but with nuchal electromyograph (EMG) and central EEG scoring, verifies that arousals indeed occur.\textsuperscript{18} Carley et al administered 4000 Hz, 103 dB, 0.5 sec duration noise, every 30 sec for 6 h during the day by a loudspeaker attached to individual Plexiglas chambers of approximately the same size as our chambers.\textsuperscript{18} The 3-sec arousals were unaffected by sleep stage, including REM sleep. The tones caused an increase in percent
wake time, no change in non-slow-wave sleep, and a decrease in slow wave and REM sleep. These authors demonstrated that on average, 65% of tones were followed by at least a 3-sec EEG desynchronization indicative of arousal. After the first hour, however, only 40% to 45% of acoustic tones (120 per hour) produced EEG arousal through the sixth hour (personal communication, D.W. Carley). Although these authors did not measure movement, their EEG habituation plateau corresponds well to our movement plateau on day one, where only 25 movements/h or 20% of tones resulted in movement after the first hour (Figure 1A). The movement plateau after the first hour drops by day 35 to 15 movements/hour or about 13% of the 120 stimuli per hour (Figure 1B). Although neither Carley et al’s data nor our study correlate EEG arousal to movement after 35 days of repetitive stimulation, it can be assumed that the number of acoustic stimuli resulting in EEG arousal still exceeded the number of movements induced by the noise stimuli, which remained consistently higher in the study versus control groups at all hours on both day 1 and day 35. Thus, while it can be argued that there was attenuation in body movement and acute BP response to noise after 35 days, the movement response and hence arousal response remained higher in the study group, yet unstimulated resting BP did not rise. Without EEG data on the number of arousals on day 35, we cannot conclude whether the lack of chronic BP response to 35 days of episodic noise stimulation was due to arousal/movement habituation to the noise or cardiovascular habituation to the arousal/movement.

Other acute noise-tactile stimulus studies confirm the rapid movement habituation. Casto and Printz used an acute air puff of 12.5 psi for 100 msec directed over the dorsum of spontaneously hypertensive rats (SHR) or WKY rats at 30 sec intervals for 30 stimuli. MAP increased acutely by 36.4 mm Hg after the first stimulus but by only 15.4 mm Hg after the 20th stimulus. This is comparable in our rats to the mean increase of 19.4 and 24.6 mm Hg (experimental versus controls) at 5 min but only 15.7 mm Hg at 30 min to the same level of stimulation. Casto et al also found a biphasic HR response (bradycardia-tachycardia) to air puff, similar to noise or episodic hypoxia stimulated rats in this and other studies. Thus, considering the findings of Carley, the current study, and others, there appears to be habituation in movement and acute cardiovascular response to noise, but no habituation of EEG arousal.

On the other hand, our results do not agree with those in other rat preparations of chronic noise stimulation. However, it is difficult to find comparable examples using the same conditions that we needed to duplicate our episodic hypoxia arousal pattern. For example, Altura et al subjected 12-week-old male Wistar-Kyoto (WKY) rats to acoustic stress consisting of 85 dB, 12 h/day, 8 pm to 8 am, for 8 weeks, then 95 dB, 16 h/day, for an additional 4 weeks. Intraarterial BP measured in unstimulated controls and experimental animals, measured only at the end of the experiment, was 132/84 mm Hg and 148/89 mm Hg, respectively (P < .01). The noise stimulus was continuous as opposed to episodic, of greater duration (12 to 16 h/day), over a more prolonged time period (12 weeks), and given during the normal waking/active period of the animal while sleep remained undisturbed. Fisher and Tucker used air jet noise (120 dB, 30 to 120 sec, 2 h/day) on first generation crosses (F1) between spontaneously hypertensive rats (SHR) and WKY rats for up to 10 weeks. There was no difference in MAP between controls and noise-stimulated rats at 2 weeks, but at 10 weeks control MAP was 128 ± 4 mm Hg vs 144 ± 4 mm Hg for stimulated rats. Again, major differences between their study and our current preparation is that the air jet is both a noise and tactile stimulus, the noise was in 30 to 120 sec pulses, the F1 is a hypertension prone rat, the duration of exposure was 10 weeks, and BP was measured between 20:00 and 07:00 when the rats are normally active, while our measurements were during the early hours of sleep (09:00 to 13:00). Also, the time of day of the chronic stimulus may be quite important, but is not mentioned. Wu et al exposed Sprague-Dawley rats to continuous noise (100 dB, 1000 Hz) for 4 h/day, 6 days/week, for 4 weeks. Systolic BP was measured by tail cuff and increased from 110 ± 5 mm Hg to 141 ± 6 mm Hg at 4 weeks. The time of day of exposure was not given. Also, the exposure was not intermittent, having been given continuously for 4 h.

Despite disagreement with the various models of chronic noise in rats, our results fit well with those of a recently published study comparing the chronic BP effects of recurrent obstructive apnea in sleeping dogs with the effects of recurrent nocturnal arousal. Brooks et al created OSA in four dogs by intermittent airway occlusion during sleep for 1 to 3 months. This regimen produced a sustained daytime MAP elevation of 15.7 mm Hg at > 4 weeks of obstruction. Six months following recovery from the apnea induced elevation of BP, an acoustic alarm (17 to 30 KHz) was used to recurrently arouse these same animals from sleep in a pattern similar to that of the induced airway obstruction, but now with normal breathing. Although acute nocturnal (sleeping) BP was elevated in response to acoustic arousal, daytime BP was not, either during or after the trial exposure. Thus with each animal as its own control, apnea with obstruction, desaturation, and arousal produced a sustained daytime elevation in BP, whereas arousal alone did not. The findings of our current study along with those of Brooks et al suggest that hypoxemia is a major inciting mechanism.
of diurnal BP elevation. This does not rule out hypercapnia as a contributing factor, although previous studies in rats using this model do not show any added effect of asphyxia on chronic BP elevations.14

REFERENCES