Protoveratrin A and B increase sleep apnea index in Sprague-Dawley rats

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Protoveratrin A and B increase sleep apnea index in Sprague-Dawley rats. J. Appl. Physiol. 83(5): 1602–1606, 1997.—The action of protoveratrin A and B, which stimulate carotid sinus baroreceptors and vagal sensory endings in the heart as well as pulmonary bed, were assessed on spontaneous and postsigh central sleep apneas in freely moving Sprague-Dawley rats. During the 6-h recording period, animals were simultaneously monitored for sleep by using electrophysiologic and electromyographic recordings, for respiration by single-chamber plethysmography, and for blood pressure and heart period by using radiotelemetry. After administration of 0.2, 0.5, or 1 mg/kg sc of protoveratrin, cardiopulmonary changes lasting at least 6 h were observed in all three behavioral states [heart period increased up to 23% in wakefulness, 21% in non-rapid-eye-movement (non-REM) sleep, and 20% in REM sleep; \( P < 0.005 \) for each]. At the same time, there was a substantial increase in the number of spontaneous (375% increase; \( P = 0.04 \)) and postsigh (268% increase, \( P = 0.0002 \)) apneas. Minute ventilation decreased by up to 24% in wakefulness, 25% in non-REM, and 35% in REM sleep (\( P < 0.05 \) for each). We conclude that pharmacological stimulation of baroreflexes promotes apnea expression in the sleeping rat.

METHODS

Ten adult male Sprague-Dawley rats (weighing 300 g) were maintained on a 12:12-h light (0800–2000)–dark (2000–0800) cycle for 1 wk, housed in individual cages, and given ad libitum access to food and water. After 1 wk of adaptation, animals were subjected to surgical procedures that are briefly described here.

Rats were anesthetized for the implantation of cortical electrodes for electroencephalogram (EEG) recording and of neck muscle electrodes for electromyogram (EMG) recording by using a mixture of ketamine (Vetalar 100 mg/ml) and acetylpromazine (10 mg/ml) (4:1, vol/vol) at a volume of 1 ml/kg body wt. The surface of the skull was exposed and cleaned with a 20% solution of hydrogen peroxide followed by a solution of 95% isopropyl alcohol. Next, a dental preparation of sodium fluoride (Flura-GEL, Saslow Dental, Mt. Prospect, IL) was applied to harden the skull and allowed to remain for 5 min. The fluoride mixture was then removed from the skull above the parietal cortex. A thin layer of J-usti resin cement (Saslow Dental) was applied to cover the screw heads and surrounding skull to further promote the adhesion of the implant. EMG electrodes consisted of two ball-shaped wires that were inserted into the bilateral neck musculature. All leads were soldered to a miniature connector (39F1401, Newark Electronics). Finally, the entire assembly was fixed to the skull with dental cement.

After surgery, all animals were allowed a 1-wk recovery before being subjected to another surgery, which involved implantation of a radiotelemetry transmitter (TA11PA-C40, Data Sciences International, St. Paul, MN) for monitoring BP and HP. After rats were anesthetized (as described above), the hair from the subxiphoid space to the pelvis was removed. The whole area was cleaned with a 20% solution of sodium fluoride and rinsed with alcohol and saline. A 4- to 6-cm midline abdominal incision was made to allow good visualization of the area from the bifurcation of the aorta to the renal arteries. A retractor was used to expose the contents of the abdomen, and the intestine was held back by using saline-moistened gauze sponges. The aorta was dissected from the surrounding fat and connective tissues by using sterile cotton applicators. A 3-0 silk suture was placed beneath the aorta, and traction was applied to the suture to restrict the blood flow. Then the implant was held by forceps while the aorta was punctured just cranial to the bifurcation by using a 21-gauge needle bent at the beveled end. The tip of the catheter was inserted under the needle by using the needle as a guide until the thin-walled BP sensor section was within the vessel. Finally, one drop of tissue sinus baroreceptors and pulmonary venous stretch receptors. The aim of this study was to test the hypothesis that baroreceptor afferent stimulation by PV inhibits respiratory drive and leads to an increased apnea expression. As an indirect sign of dose-dependent baroreflex stimulation by PV, our results show the dose-related effect of the drugs on heart period (HP). This effect was paralleled with an inhibition of breathing, resulting in an increased sleep apnea index.
adhesive (Vetbond, 3M) was applied to the puncture site and covered with a small square of cellulose fiber (~5 mm²) for sealing the puncture after catheter insertion. The radio implant was attached to the abdominal wall by 3–0 silk suture, and the incision was closed in layers.

After the second surgery, animals were allowed a 1-wk recovery period before being used in the study. Each rat was recorded on seven occasions: control (saline), three doses of PVA (0.2, 0.5, and 1 mg/kg) and three doses of PVB (0.2, 0.5, and 1 mg/kg). These doses are very similar to those reported to elicit significant bradycardia and hypotension (27). All doses were applied by subcutaneous injection at 0945. Polygraphic recordings were made from 1000 to 1600 and were separated by at least 3 days.

Respiration was recorded by placing each rat, unrestrained, inside a single-chamber plethysmograph (PLYUN1R; Buxco Electronics, Sharon, CT; dimensions: 6-in. width × 10-in. length × 6-in. height) ventilated with a bias flow of fresh room air at a rate of 2 l/min. A cable plugged onto the animal’s connector and passed through a sealed port was used to carry the bioelectrical activity from the head. Respiration, BP, 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 by using the biparietal EEG and nuchal EMG signals on 10-s epochs, as described by Bennington et al. (6) (the software was kindly provided by J. Bennington). 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. Sleep efficiency was measured as the percentage of total recorded epochs staged as NREM or REM sleep.

As in previous investigations (9–12, 28, 29), sleep apneas, 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, i.e., NREM or REM sleep. The duration requirement of 2.5 s represents at least two “missed” breaths, which is, therefore, analogous to a 10-s apnea duration requirement in humans, also reflecting 2–3 “missed” breaths. The events detected represent central apneas, because decreased ventilation associated with obstructed or occluded airways would generate an increased plethysmographic signal rather than a pause. As in previous reports, apneas were observed to occur either as pauses between breaths (spontaneous (Sp)) or as periods of respiratory cessation preceded by a sigh (postsigh (PS)). Therefore, we characterized them as PS apneas and Sp apneas according to the presence or absence of a preceding inspiration at least 150 ms longer than the average tidal amplitude during regular breathing. An apnea index (AI), defined as apneas per hour in a stage, was separately determined for NREM and REM sleep. Comparison of AI between rats treated with PVA and PVB was made on the group mean data by using analysis of variance (ANOVA). The effects of sleep stage (NREM vs. REM) were made by using ANOVA with repeated measures. Multiple comparisons were controlled by using Fisher’s protected least significant difference. In addition, the timing and volume of each breath were scored by automatic analysis (Experimenter’s Workbench; Datawave Technologies, Longmont, CO). For each animal, the mean respiratory rate (RR) and minute ventilation (Vi) were computed for W during the

control recordings and used as a baseline to normalize respiration during sleep and during PV administration in a given animal. One-way ANOVA was also performed by nonparametric (Kruskal-Wallis) analysis. Conclusions based on parametric and nonparametric ANOVA were identical in all cases.

Similar software was employed to analyze the BP waveform; for each beat of each recording, systolic (SBP) and diastolic (DBP) BP values and pulse interval were measured. The pulse interval provided a beat-by-beat estimate of HP. Mean BP (MBP) was estimated according to the weighted average of SBP and DBP for each beat: MBP = DBP + (SBP – DBP)/3. The parameters for each beat were also classified according to the sleep/wake state and recording hour during which they occurred.

RESULTS

Figure 1 shows that PV did not affect MBP during NREM sleep. In addition, PV did not affect MBP during W or REM sleep (P > 0.7 for all) (data not shown). The dose-dependent effects of these two compounds on cardiovascular, respiratory, and state variables were assessed by ANOVA. PVA and PVB had equivalent effects on all variables except HP. For this reason, PVA and PVB data were pooled for all analyses, except as presented in Figs. 2 and 3.

Figure 2 illustrates the effects of 0.2, 0.5, and 1.0 mg/kg of PVA and PVB on HP during NREM sleep. Administration of PVA and PVB produced an increase in HP during NREM sleep by up to 17%, ranging from 166 to 200 ms (P < 0.03 to P < 0.001). Administration of PV also produced an increase in HP during W state by 19%, from 154 to 188 ms (P < 0.0002), and during REM sleep by 25%, from 174 to 205 ms (P < 0.008) (data not shown).

Figure 3 presents the dose-dependent effects of PVA and PVB on HP in individual animals. It shows that the observed group mean effects (Fig. 2) were, in fact, reflected by virtually every individual animal.

The effects of PV on Vi during NREM sleep are illustrated in Fig. 4. Fisher’s analysis showed that doses of 0.5 and 1 mg/kg of PV suppressed Vi by 22 and

Fig. 1. Effects of 0.2, 0.5, and 1.0 mg/kg of protoveratrine (PV) on mean blood pressure during non-rapid-eye-movement (NREM) sleep. Data reflect pooled analysis of means ± SE for PV A and B (PVA and PVB, respectively) (see text for details). C, saline control.
25% compared with control (P = 0.04 and P = 0.01, respectively). It is noteworthy that, although dose profiles for the V˙I effect of PVA and PVB were statistically equivalent, the numerical effects were analogous to those observed for HP; i.e., the decrement in V˙I for the 0.2 mg/kg dose of PVA was only 5% (P > 0.5 vs. control), whereas the decrement for 0.2 mg/kg of PVB was 22% (P < 0.05). Thus PVB did not exhibit a significant dose response on HP or V˙I for the concentrations tested. Administration of PV also suppressed V˙I during W by 25% compared with control (P = 0.008) and during REM sleep by up to 35% compared with control (P = 0.0005; data not shown).

The effects of 0.2, 0.5, and 1.0 mg/kg of PV on Sp apneas during NREM sleep are shown in Fig. 5. The administration of 0.5 mg/kg of PV increased Sp index almost fourfold (P = 0.02), and administration of 1.0 mg/kg of PV increased Sp index almost fivefold (P = 0.001) in comparison with baseline. There was a similar effect of PV on Sp index during NREM sleep, which increased almost threefold with the 0.5 mg/kg dose (P = 0.002) and almost fourfold with the 1.0 mg/kg dose (P < 0.0001) compared with baseline (data not shown). The administration of PV did not affect either PS or SpAl in REM sleep (P = 0.466 and 0.760, respectively; data not shown).

Figure 6 depicts the relationship between changes in V˙I and apnea expression during NREM sleep. The y-axis describes total apneas per hour (Sp+PS), and the x-axis relates V˙I relative to control wakefulness in each animal. Seven points are plotted for each animal, corresponding to control and three doses of each PV compound. The correlation between decreased V˙I and increased Al is readily apparent in that 9 of the 10 highest Al values correspond to 9 of the 10 greatest reductions of V˙I (the vertical line in Fig. 6 demarcates the lowest 10 V˙I observations, whereas the horizontal
line segregates that highest 10 AI values). It is of note that 5 of the 10 highest AI values were associated with less than the highest dose of PV. Figures 5 and 6 also demonstrate the degree to which apnea expression can be increased when V˙I is sufficiently suppressed: AI > 45 represents a >600% increase with respect to control recordings. This apnea promotion is even more apparent when the temporal course of the response is considered.

DISCUSSION

The present results demonstrate for the first time that pharmacological stimulation of baroreflexes in the rat is associated with increased apnea expression. Administration of 1 mg/kg of PV increased Sp index almost fivefold in comparison with control, whereas PS index increased almost fourfold. The strong correlation between increased AI and decreased V˙I supports the theory that one mechanism underlying central apnea is reflex inhibition of the respiratory center (8).

We used doses of PV similar to those reported by Nagaoka (27) in unanesthetized Wistar and SHR rats by using the tail cuff method to determine a hypotensive effect. We did not observe hypotension. Two-way ANOVA demonstrated that the effects of PVA and PVB were equivalent for DBP, SBP, pulse, and MBP in W state and in NREM and REM sleep. There were no differences between the effects of PVA and PVB, and there was also no interaction with the dose for any of these variables.

With respect to HP, there was a slight but significant difference between the two compounds; PVB led to maximal lengthening of HP even at the lowest dose, whereas PVA led to a gradual dose-dependent increase in HP to the same maximal level as PVB. This distinction between PVA and PVB was present in all three behavioral states. The dose-dependent increase in HP may be viewed as a marker of baroreflex stimulation (21). The fact that BP did not decrease indicates the presence of compensatory increases in stroke volume, vascular resistance, or both. The absence of hypotension reduces the likelihood that the observed respiratory effects were due to nonspecific circulatory changes. The present findings also suggest, therefore, that hy- dralazine-induced hypotension most probably suppresses apnea (11) by decreasing baroreceptor stimulation, rather than indirectly, as a result of hypotension per se.

As for BP, two-way ANOVA demonstrated equivalence of PVA and PVB for effects on RR, V˙I, and AI values, leading to the pooled analysis presented. With respect to apnea expression, we observed a striking state dependence: PV dramatically potentiated NREM apneas without altering REM apneas in any significant way. Yet, the effects of PV on HP and V˙I were similar in NREM and REM sleep. Thus REM sleep reveals a divergence between a suppression of V˙I and increased apnea expression. Apnea genesis in the rat, as in human, appears to be multifactorial, and it is too simple to conclude that anything affecting V˙I will affect apnea expression.

The present results are consistent with our previous investigations in which we were not able to modulate apnea expression in REM sleep with hydralazine (11) or adenosine agonists N6-p-sulfophenyladenosine (26), R(-)IN6-L-(2-phenylisopropyl)labeledenosine or CGS-21680 (25). We were able to suppress REM sleep apneas using hypercapnia or hypoxia (13). Taken collectively, these studies indicate that it is more difficult to alter apnea expression via peripheral reflexes during REM than during NREM sleep. This is consistent with many studies demonstrating dampening or ablation of various peripheral inputs during REM sleep (14). Because PV compounds will stimulate all exposed nerve endings (5), the observed effects may not have resulted directly or exclusively from baroreflex stimulation. Other, unmeasured but possibly relevant, reflexes may have been more attenuated during REM sleep than was the chronotropic baroreflex.

PVA and PVB may have had direct central nervous system effects. There are changes in the sleep architecture, but they do not necessarily imply central action, since there is evidence that stimuli originating in the periphery, including baroreflex stimulation (4), can alter sleep architecture. There are also proposed central baroreceptors similar to those of the carotid sinuses (30). The central effect of PV on respiration can be either stimulatory, possibly through medullary areas (accessible to cisternal or vertebral injection), or inhibitory, through another intracranial area, possibly meningeal (5). However, regardless of the action, central or peripheral, it is known that PV stimulates baroreceptor nerve endings. There is also evidence of

Table 1. Effect of PV on apnea duration

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.2 mg/kg</th>
<th>0.5 mg/kg</th>
<th>1.0 mg/kg</th>
</tr>
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<tbody>
<tr>
<td>Spontaneous</td>
<td>3.2 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>Postsigh</td>
<td>3.4 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
</tbody>
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Values are means ± SE. Apnea duration is equivalent to control (P > 0.3) for spontaneous and postsigh apneas at all protoveratrine (PV) doses.
The direct involvement of baroreceptor afferent firing in apnea genesis (31).

The absence of PV effect on apnea length supports the conclusion that apnea initiation and apnea resolution are independently controlled events. The dissociation between apnea initiation and apnea maintenance is particularly clear in the first hour after injection, during which Al can be increased as much as 8- (for Sp apneas) to 40-fold (for PS apneas), with no effect on apnea duration. The duration of Sp and PS apneas was unaffected by PV at any dose (see Table 1).

In summary, we have shown that the PVs have a potent apnea-promoting action during NREM but not during REM sleep in rats. We further demonstrated a lack of PV effect on apnea duration and concluded that apnea initiation and apnea maintenance are controlled separately. PV administration also led to clear stimulation of the chronotropic baroreflex without change in BP, suggesting that baroreflexes play an important role in apnea genesis in the rat.

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