Desipramine increases genioglossus activity and reduces upper airway collapsibility during non-REM sleep in healthy subjects.

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ONLINE DATA SUPPLEMENT

SUPPLEMENTAL METHODS

Anthropometric data. Circumference of the neck was measured at the superior border of the cricothyroid cartilage, waist circumference was measured at the highest point of the iliac crest.

End tidal O_2 and CO_2. Exhaled oxygen and carbon dioxide was continuously recorded from a catheter placed inside the nostril and measured with a O_2 analyzer/capnograph (Vacumed, Ventura, CA) yielding end-tidal values.

Standard polysomnography montage. Subjects were instrumented for standard polysomnography (PSG) equipment; electroencephalography (EEG), chin electromyography (EMG), and electrooculography (EOG) were recorded for sleep staging. Piezo-electric bands around the chest and abdomen monitored respiratory movements. Electrocardiography body position and arterial oxygen saturation were also measured.

Genioglossus electromyography measurements (EMG_{GG}).

EMG_{GG} was recorded using unipolar intramuscular electrodes at a sampling frequency of 1000 Hz. The electrodes consisted of two stainless steel Teflon-coated 30-gauge wires that were inserted orally approximately 15 mm into the body of the genioglossus muscle. All data were acquired using Spike 2 software with a 1401 interface (Cambridge Electronic Design, Cambridge, UK). All polysomnography and EMG signals were amplified and filtered using GRASS amplifiers (Grass Telefactor, West Warwick, RI) prior to analogue-to-digital conversion (ADC) by the 1401 interface.

The raw EMG_{GG} was band-pass filtered (between 30 Hz and 1 kHz), rectified, and integrated using a 100 ms window. A notch-type filter (50/60 Hz) was also applied to reduce the signal noise.

SUPPLEMENTAL RESULTS

Effect of desipramine on genioglossus electromyography (EMG_{GG}) during sleep: alternative statistical analysis. The power analysis of EMG_{GG} pilot data was based on the assumption of a normal distribution, however we performed a Wilcoxon matched-pairs signed rank test in the trial to analyse the difference in EMG_{GG} between placebo and desipramine nights, in order to reduce the impact of outliers in this small group of subjects. To avoid the possible criticism that the statistical methodology had changed from a pre-trial specification, we performed a paired T test to determine if difference in EMG_{GG} were still present assuming a normal distribution.

We found no difference between the two statistical tests. Tonic EMG_{GG} activity was higher during NREM sleep on desipramine compared to placebo (104±22 vs. 72±24 $\%_{wake}$, p=0.003). Phasic EMG_{GG} activity (peak minus tonic) was not consistently altered by desipramine (76±37 on placebo vs. 94±53 $\%_{wake}$ on desipramine, p=0.37). Results are expressed as mean±standard deviation.

Effect of desipramine on wakefulness EMG_{GG}. We sought to address the possibility that desipramine may have influenced EMG_{GG} during wakefulness. Of note, wakefulness $EMG_{GG,}$ tonic, and phasic values were similar between nights (tonic 2.3 [2.0; 6.7] on placebo vs. 2.1 [1.7;

2.6]AU on desipramine, p=0.19; phasic 1.1 [0.5; 2.8] on placebo vs 1.6 [0.8; 3.1]AU on desipramine, p>0.5).

The number of breaths analyzed per person during wakefulness was 94±49 on the placebo night and 102±78 during the desipramine night.

Muscle responsiveness. The responsiveness of EMG_{GG} phasic activity to negative pharyngeal pressure is attenuated from wakefulness to sleep (1). We hypothesized that desipramine might also increase muscle responsiveness to progressively-greater epiglottic pressure swings. Contrary to the basal genioglossus activity, genioglossus responsiveness was not influenced by desipramine (-0.42 [-0.16; -0.96] on placebo vs. -0.48 [-0.12; -1.18] AU/cmH_2O on desipramine, p>0.5).

Post hoc analysis: effects of desipramine on EMG_{GG} activity during CPAP manipulation. We performed a post-hoc analysis to evaluate the effect of desipramine on EMG_{GG} activity during optimum CPAP, during rapid CPAP dial downs, and at the end of slow CPAP dial downs to investigate the possible link between increased EMG_{GG} activity and reduced upper airway collapsibility on desipramine. The average optimum CPAP on both placebo and desipramine nights was 3.5±3.2 cmH2O in these healthy controls. Measurements were performed during sleep in the supine position but referred to baseline wakefulness on the side. EMG_{GG} measurements during CPAP manipulation were not matched with wakefulness epiglottic pressure swings. We found that on optimum CPAP in the supine position, there was no difference in tonic (86 [52; 98] on placebo vs 88 [84; 92]$\%_{wake}$ on desipramine, p=0.16) or phasic activity (66 [54; 90] on placebo vs 66 [38; 96]$\%_{wake}$ on desipramine, p=0.65). However on

desipramine, compared to placebo, tonic EMG_{GG} activity was higher immediately following rapid CPAP dial downs (83 [61; 88] on placebo vs 101 [88; 160] $\%_{wake}$ on desipramine, p=0.049) while there was a trend for reduced phasic activity (102 [66; 155] on placebo vs 53 [29; 79] $\%_{wake}$ on desipramine, p=0.11). During slow CPAP dial downs there was a trend for a higher tonic activity (111 [101; 217] on placebo vs 205 [225; 90]$\%_{wake}$ on desipramine, p=0.11) and lower phasic activity on desipramine (213 [102; 319] on placebo vs 111 [62; 163]$_{\%wake}$ on desipramine, p=0.11). These data suggest that desipramine increased the stiffness of the upper airway primarily by enhancing tonic activity of upper airway muscles.

Learning effect. In order to test for any learning effect, we compared sleep architecture (total sleep time, non-REM stage 1, 2, 3 and REM sleep time) and physiology measurements (EMG_{GG}, Pcrit) between night 1 and night 2 and we found no statistically significant difference ($p>0.5$) for all these parameters. Moreover, to test for a treatment vs treatment order interaction on the primary and secondary outcomes (EMG_{GG} and Pcrit, respectively) we compared the average of the measurements on the two treatments between allocation orders (i.e. placebo first vs desipramine first) and we did not find any significant difference between groups ($p>0.5$), excluding learning effects from first to second treatment assessment.

SUPPLEMENTAL DISCUSSION

Comment on the lack of effect of desipramine on muscle responsiveness and inspiratory phasic EMG activity. During a study conducted in OSA patients, Berry and colleagues (2) found

that administration of a selective serotonin reuptake inhibitor (paroxetine 40 mg) increased phasic EMG_{GG} activity and responsiveness to negative pressure during obstructive events but did not affect tonic genioglossus activity. Given that we found no increase in muscle responsiveness in the current study, we could speculate that different neurotransmitters could activate different motoneuron pools, with noradrenergic activation mainly linked to tonic genioglossus activation while serotonergic activity specifically stimulates inspiratory activity and muscle responsiveness.

About a possible relationships between change in sleep architecture and changes in EMG_{GG} or Pcrit. Changes in NREM sleep architecture with desipramine could have affected our results, e.g., different percentages of stage N1, N2 or N3 could be responsible for differences in EMG activity and collapsibility measurements. It has been shown in multiunit (3) and single motor unit (4) EMG_{GG} recordings that genioglossus activity is higher during NREM sleep stage N3 compared to other NREM stages. Likewise, recently Carberry et al. (5) found that the Pcrit during stage N3 is slightly lower (upper airway less collapsible) than in N2. However, we think this is unlikely to have affected the interpretation of our findings for the following reason. If sleep stage had an impact on our EMG_{GG} and collapsibilty findings, this effect would have tended to reduce EMG_{GG} activity and increase upper airway collapsibility observed on the desipramine night, where there was a trend towards a reduced N3. This effect would likely have masked our findings that EMG_{GG} was higher and the upper airway was less collapsible on desipramine.

About anticholinergic effects of desipramine. Although not assessed with our experimental setup, the effect of desipramine on Pcrit might also be associated with the anticholinergic properties of desipramine which can cause mouth dryness, potentially reducing upper airway edema (6) or surface adhesion forces (7).

Potential limitations of EMG data comparison between nights. Raw EMG amplitude may vary with the electrodes and their site of insertion, making comparisons of EMG amplitude between nights challenging. To reduce the EMG_{GG} variability due to these technical issues, we normalized the sleep values by the baseline wakefulness values. Comparison of wakefulness values is more problematic as the measurements were expressed in arbitrary units (moving time average of 0.1 sec from raw data). For these reasons, wakefulness data need to be interpreted with caution. Nevertheless, we found that their distribution was very similar between nights, suggesting that desipramine may have no substantial effect on wakefulness EMG_{GG}.

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