Differential suppression of upper airway motor activity during carbachol-induced, REM sleep-like atonia

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Fenik, Victor, Richard O. Davies, Allan I. Pack, and Leszek Kubin. Differential suppression of upper airway motor activity during carbachol-induced, REM sleep-like atonia. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1013–R1024, 1998.—Microinjections of carbachol into the pontine tegmentum of decerebrate cats have been used to study the mechanisms underlying the suppression of postural and respiratory motoneuronal activity during the resulting rapid eye movement (REM) sleep-like atonia. During REM sleep, distinct respiratory muscles are differentially affected; e.g., the activity of the diaphragm shows little suppression, whereas the activity of some upper airway muscles is quite strong. To determine the pattern of the carbachol-induced changes in the activity of different groups of upper airway motoneurons, we simultaneously recorded the efferent activity of the recurrent laryngeal nerve (RL), pharyngeal branch of the vagus nerve (Phar), and genioglossal branch of the hypoglossal (XII) and phrenic (Phr) nerves in 12 decerebrate, paralysed, vagotomized, and artificially ventilated cats. Pontine carbachol caused a stereotyped suppression of the spontaneous activity that was significantly larger in Phar expiratory (to 8.3% of control) and XII inspiratory motoneurons (to 15%) than in Phr inspiratory (to 87%), RL inspiratory (to 79%), or RL expiratory motoneurons (to 72%). The suppression in upper airway motor output was significantly greater than the depression caused by a level of hypocapnia that reduced Phr activity as much as carbachol. We conclude that pontine carbachol evokes a stereotyped pattern of suppression of upper airway motor activity. Because carbachol evokes a state having many neurophysiological characteristics similar to those of REM sleep, it is likely that pontine cholinoreceptive neurons have similar effects on the activity of upper airway motoneurons during both states.

SLEEP, PARTICULARLY the rapid eye movement (REM) stage, exerts profound effects on the control of all skeletal muscles. One of the major signs of REM sleep is a strong suppression of postural muscle tone. In parallel with this postural atonia, there are decreases in the tone of both respiratory pump and upper airway muscles, the changes in upper airway muscle activity frequently being large and leading to airway narrowing and, in predisposed individuals, to sleep-disordered breathing (5, 21, 38, 47). The suppression of upper airway activity is not uniform, with the decrease of activity in most pharyngeal dilators being greater than in laryngeal muscles (9, 10, 21, 40).

To study the changes in upper airway muscle activity that accompany REM sleep and the neuronal mechanisms underlying these changes, we adapted the carbachol model of REM sleep (see Refs. 15, 25, 42, and 45 for reviews). Microinjections of carbachol, a cholinergic agonist, into the pontine tegmentum of chronically instrumented, behaving cats evoke a state having many characteristics similar to those of REM sleep and consistently evoke a generalized postural atonia. The similarities between the effects of carbachol in behaving cats and REM sleep were summarized recently in reviews by Baghdoyan (see Table 1 in Ref. 2) and Lydic (see Table 1 in Ref. 25). Important for the present studies, in acutely decerebrated cats, intracellular recordings from lumbar motoneurons show that injections of carbachol into the pons induce an atonia with state-specific inhibitory postsynaptic potentials that closely resemble those recorded during REM sleep in chronic cats (31). In acutely decerebrate cats, carbachol also evokes eye movements (44) and a reduction of the firing of brain stem serotonergic raphe neurons, similar to that during REM sleep (48). Thus pontine carbachol injections can be used in acutely decerebrate cats to produce, at controlled times, a depression of motor output that is similar to the atonia of REM sleep. Consequently, this preparation provides an opportunity to study the central neuronal mechanisms that underlie the REM sleep-like depression of motor activity with the use of neurophysiological and neuropharmacological techniques whose application in chronic animal studies is difficult to implement.

We previously studied the effects that microinjections of carbachol into the pons of decerebrate cats have on selected respiratory motoneuronal outputs and found a stereotyped, differential suppression of the motoneurons to respiratory pump muscles, with the following increasing order of the magnitude of suppression: diaphragm < inspiratory intercostals < expiratory intercostals (13, 44). In addition, we studied the motoneuronal activity of one pharyngeal dilator muscle, the genioglossus, whose carbachol-induced suppression was very profound (to 10% of control) (13). This and observations of the activity of selected upper airway muscles during REM sleep in humans (20, 23, 40, 47) and experimental animals (10, 27, 41) showed that large differences exist in the magnitude of suppression among distinct upper airway muscles. However, a clear pattern of those changes is difficult to discern based on the existing body of evidence. Because the pharynx is the region most vulnerable to airway obstruction during sleep (9, 21, 38, 40), we hypothesized that the carbachol-induced suppression of activity would be stronger in pharyngeal than in laryngeal motoneuronal groups. The specific aim of the study was to test this hypothesis by simultaneously measuring the carbachol-induced changes in the motor activity of selected nerves inner-
vating these structures. We used paralyzed, vagoto-
mized, and artificially ventilated decerebrate cats to
ensure that the carbachol-induced changes in neuronal
activity were due solely to the activation of central
cholinceptive mechanisms in the pons and were not
confounded by peripheral feedbacks.

METHODS

Animal preparation. The results reported are from experi-
mements on 12 cats of either sex (2.5–3.8 kg) preanesthetized
with ketamine (100 mg im) and diazepam (2 mg im), intuba-
ted, anesthetized with halothane (0.2–1.0%), and decer-
ебated at a precolluceral level (14). Anesthesia was then
discontinued. The procedures used to minimize animal discom-
fort, for anesthesia, for decerebration, and for all subsequent
preparations of the animal and recording were approved by
the Institutional Animal Care and Use Committee of the
University of Pennsylvania. The surgical methods and record-
ing techniques have been described in detail previously (13).
Recordings began ~9 h after the injection of ketamine-
diazepam and 7 h after the decerebration (half-life of diaz-
epam in the cat is ~5 h; thus residual effects of drug were
present throughout experiment).

A femoral artery and vein were catheterized for blood
pressure recording and drug administration, respectively.
The C5 branch of the phrenic nerve (Phr), a dorsal motor
pressure recording and drug administration, respectively.

TABLE 1. Patterns of spontaneous activity in upper airway nerves

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Animal Number</th>
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<tr>
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<td>37 (2)</td>
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<td>RL</td>
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<td>40 (2)</td>
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<td>53 (2)</td>
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<td>54 (1)</td>
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Numbers in parentheses indicate no. of carbachol microinjections performed in each animal; in some experiments, effect of carbachol was
reversed by a pontine microinjection of atropine and a second injection of carbachol was made in the contralateral pons. RL, recurrent
laryngeal nerve; XII, hypoglossal nerve; Phar, pharyngeal branch of the vagus nerve. Activity patterns: I-Aug, inspiratory augmenting; E-Dec,
extpiratory decrementing; C-L, coughlike bursts; NA, no activity recorded and/or analyzed in that nerve during the given experiment.
decrease in nerve activity occurred followed by a steady state. Atropine was then used to cause a recovery from the effects of carbachol. In the cat, within the time frame of an acute experiment, one can produce only two responses to carbachol, one from each side of the pons (13). We made 18 carbachol injections in 12 animals: 12 first injections and 6 second injections. The data from each carbachol injection were considered as a separate test. In Fig. 4, we indicate which data sets are from animals with two carbachol injections and whether the given set is from the first or second injection.

After 10 of the carbachol injections (7 animals, with 7 first injections and 3 second), atropine was microinjected into the pons and caused a recovery from the effect of carbachol. For the atropine microinjections, the carbachol pipette was removed and replaced with one containing atropine, positioned at the same coordinates. In six of the seven animals in which the first injection of carbachol was followed by a pontine microinjection of atropine that caused a full recovery of activity, a second carbachol microinjection was made in a symmetrical site on the left side, using the postatropine conditions as a control. In the remaining animal, the effect of the first injection of carbachol was reversed by a pontine microinjection followed by an intravenous injection of atropine (1 mg/animal) and no second injection of carbachol was made. For the remaining carbachol injections (5 animals, with 5 first injections and 3 second injections), atropine was injected intravenously rather than in the pons to fully antagonize the effects of the second carbachol injection.

In three animals, the $P_{CO_2}$ was reduced by turning off the inspired $CO_2$ to reduce the amplitudes of the recorded nerve activities. When the amplitude of the phrenic activity equaled the phrenic amplitude present after the carbachol-induced suppression, the pattern of the resulting decreases in upper airway nerve activity was compared with that produced by carbachol. The $CO_2$ level was then restored to control value.

Data analysis. All analyses were performed off line using a personal computer-based data acquisition and analysis system (EGAA, RC Electronics) and graphics and statistics software (SigmaPlot and SigmaStat, Jandel). From the moving average of the phrenic neurogram, we determined the onset of each inspiration and expiration during the period that was analyzed; these were used to calculate the respiratory rate and the duration of inspiration (Ti) and expiration (Te). The mean amplitude of the peak inspiratory and/or expiratory activity of each nerve was measured from the moving average 1) during the control period, 2) during the period of maximum suppression of activity after the carbachol injection, 3) after the recovery produced by atropine, and 4) after decreasing the $P_{CO_2}$. Cycle-triggered averaging of nerve activity was performed during steady-state periods using the onset of expiration as a trigger. The baseline of the moving average of the nerve activities was established from those segments of the recording when there were no spikes during a part of the respiratory cycle, as verified by observation of the raw nerve activity. For each experiment, measurements of the various parameters of the responses were normalized relative to the mean control values immediately before each carbachol microinjection. The mean number of respiratory cycles analyzed to derive the average steady-state control values was $51 \pm 7.0$ (range of 10–134 cycles, determined by available duration of data on tape).

Two-tailed Student’s paired or unpaired t-tests with Bonferroni correction were used for statistical comparisons. Differences were considered significant when $P < 0.05$. The variability of the means is expressed by the standard error throughout this report.

RESULTS

Patterns of spontaneous activity under control conditions. In addition to the Phr and Phar, whose activities were recorded in all animals, stable respiratory-modulated activity having a consistent pattern in control conditions was recorded in the RL in 9, and XII in 10, of the 12 animals. The upper airway nerve activities had a stable pattern in each animal, but, across different animals, some components were consistently present, whereas others were variable. Only those components consistently present were analyzed. Table 1 summarizes the details of the patterns seen in each animal and nerve.

The main component of RL activity had an inspiratory augmenting pattern (I-Aug) in all nine animals (see Figs. 1B and 2). In addition, in seven of the nine animals, there was early expiratory activity with a decrementing pattern (E-Dec). The latter activity started at the onset of expiration and then either slowly declined throughout this phase until the inspiratory onset (long-lasting E-Dec; 4 animals) or ceased before the end of expiration (short-lasting E-Dec; 3 animals) (see Figs. 1B and 2). Of the remaining two animals, one had augmenting activity throughout expiration (E-Aug), and the other had a steady level of expiratory activity above that of the baseline (Tonic).

The activity of XII was principally I-Aug (all 10 animals) (see Figs. 1B and 2). In addition, an expiratory component occurred with a short-lasting E-Dec pattern (3 animals) and/or a Tonic pattern (3 animals).

The activity of Phr was predominantly expiratory. In 11 of the 12 animals, it had a late E-Aug pattern (see Fig. 1B); in the remaining animal, animal 37 (Table 1), it had a long-lasting E-Dec pattern. In addition to this main expiratory component, seven animals had a small component of I-Aug activity (see Fig. 1B), three had a distinct initial peak of short-lasting E-Dec activity (see Fig. 1B), and one had a long-lasting E-Dec pattern.

In addition to the phasic activity occurring with the respiratory rhythm, in four animals there were intermittent, long-amplitude bursts of activity having a stereotyped pattern and position within the respiratory cycle. The bursts started at the onset of expiration and subsequently declined with a fixed temporal sequence in RL, XII, and Phr (see Fig. 6 and Table 1). Because they resembled the activity observed during spontaneous or reflex evoked coughs recorded from upper airway nerves by others (7, 33; see Ref. 46 for review), we refer to them as coughlike bursts.

Carbachol-induced suppression of respiratory activity. After a variable latency (range 0.5–14 min; mean 3.8 ± 1.2), pontine microinjections of carbachol always caused a suppression of spontaneous activity in all upper airway nerves, which developed with a similar time course in all nerves. This suppression was accompanied by small decreases in Phr activity in 15 out of 18 injections and in arterial blood pressure in 17 out of 18 injections. A typical response (animal 46; see Table 1) is shown in Fig. 1A. Figure 1B illustrates the patterns of nerve activity in selected segments of the same record.
on an expanded time scale during the control period, after carbachol microinjection, and after the recovery produced by pontine microinjections of atropine.

After carbachol, the activity of all nerves remained depressed for at least 10 min; then a slow, spontaneous recovery began, which could be accelerated by injecting atropine into the site where carbachol was injected earlier (Fig. 1A). The time course of the recovery produced by pontine atropine, although typically less rapid than the depressant response to carbachol, was always similar in all nerves. All intravenous injections of atropine resulted in the reappearance of all those components of nerve activity that were depressed by carbachol, thus demonstrating the cholinergic nature of the depression. However, Phr activity was often reduced after intravenous atropine, and the increases in respiratory rate were more prominent than those after pontine atropine, suggesting that atropine exerted additional central effects beyond the pontine region affected by carbachol. Therefore, quantitative data about changes in nerve activities after intravenous atropine are not included in this report.

The relative magnitude of the carbachol-induced depression of the main component of each nerve's activity was always larger in XII and Phr than in RL and Phr (Fig. 1A). In addition, distinct components of each nerve's activity were suppressed to different degrees. In Fig. 1B, both the I-Aug and the short-lasting E-Dec component of RL activity were moderately suppressed after carbachol. The I-Aug activity in XII was more strongly suppressed than that of the RL. Of the three components that could be distinguished in the control Phar activity, the E-Aug and the small, short-lasting E-Dec were abolished, whereas the small I-Aug component remained intact or slightly increased. After the pontine injection of atropine, the main components of RL, XII, and Phar activity recovered to a level greater than that before carbachol.

To assess the magnitude of the pontine carbachol- and atropine-induced changes in the activity of the different nerves, the main component(s) of each nerve's activity was measured from the cycle-triggered averages under each condition (Fig. 2). For RL, the amplitudes of the I-Aug activity and E-Dec activity were measured. For Phar, the amplitude of the E-Aug activity was measured after the 16 carbachol injections in 11 animals in which such a pattern was present. For Phr and XII, only the amplitudes of their I-Aug activities were measured.
Carbachol-induced depression of spontaneous cough-like activity. In four animals, short, stereotyped bursts of activity were generated spontaneously in the upper airway nerves at the time of inspiratory offset (Fig. 6; see also Table 1). In an individual animal, the bursts occurred fairly regularly, albeit, among animals, the interburst intervals ranged from 2 to 27 respiratory cycles. The amplitude of the bursts relative to the main respiratory components of activity was always the largest in Phar. Their duration, as measured from Phar activity, ranged from 390 to 520 ms (mean 435 ± 29 ms; n = 4).

The consistency of the overall pattern of the carbachol-induced suppression is illustrated in Fig. 4. The data are presented in their rank order according to the change of Phar activity after the carbachol injection relative to control (100%). In all but one case, the activities of RL and Phar after carbachol were >60% of control, whereas those of XII and Phar were always <40% of control.

In seven animals, the recovery from the effect of the first, and then second (n = 3), injection of carbachol was produced by pontine injections of atropine. After a new steady state was reached, the nerve activities were in most cases greater than those measured in the precarbachol control state (see Figs. 1 and 2): Phar, eight of ten trials; XII, eight of nine trials; RL-Aug, and RLE-Dec, seven of seven trials; and Phar-E-Aug, six of nine trials. Figure 5 shows the mean amplitudes of the postcarbachol and postatropine activities in each nerve relative to the precarbachol control amplitude (100%). For these 10 trials, the amplitude of Phar activity after carbachol was at 84 ± 4.9% of the precarbachol control (n = 10, P < 0.01), and after atropine it increased to 136 ± 17% of the precarbachol control (n = 10, P < 0.05); for RL-Aug the amplitude was 75 ± 4.8% (n = 7, P < 0.01) and 113 ± 3% (n = 7, P < 0.01), respectively; for RLE-Dec the amplitude was 70 ± 7.3% (n = 6, P < 0.01) and 169 ± 18% (n = 6, P < 0.01), respectively; for XII-Aug, the amplitude was 15 ± 4.4% (n = 9, P < 0.001) and 204 ± 52% (n = 9, P < 0.01), respectively; and for Phar-E-Aug the amplitude was 9 ± 4.1% (n = 10, P < 0.001) and 144 ± 39% (n = 10, P > 0.1), respectively. Thus only the postatropine overshoot of Phar-E-Aug activity did not reach statistical significance.

Figure 3 shows the mean magnitudes of the analyzed components of respiratory activity of each nerve after pontine carbachol, expressed relative to the magnitude of the corresponding component in the control condition (100%). After carbachol, the magnitude of the activity of all nerves was significantly less than control. Mean Phar activity decreased to 87 ± 3.0% of control (n = 18 injections, P < 0.001), RL-Aug activity decreased to 79 ± 3.1% (n = 13, P < 0.001), RLE-Dec activity decreased to 72 ± 4.7% (n = 10, P < 0.01), XII-Aug activity decreased to 15 ± 3.2% (n = 15, P < 0.001), and Phar-E-Aug activity decreased to 8.3 ± 2.9% (n = 16, P < 0.001). The mean arterial blood pressure decreased by 12 ± 1.8 mmHg (n = 18), corresponding to a relative mean decrease to 89 ± 1.3% of control (n = 18, P < 0.001). The mean T_i was 3.1 ± 0.5 s (median = 5.3 s) during the control period and 3.2 ± 0.6 s (median = 6.6 s) after carbachol, and the mean T_e was 5.1 ± 0.4 s (median = 5.8 s) during control and 5.9 ± 0.6 s (median = 8.3 s) after carbachol. The T_i and T_e values and their differences before and after carbachol were not distributed normally and, therefore, were analyzed after a logarithmic conversion. After the carbachol injections, log T_i did not change significantly (n = 18, P > 0.9), whereas log T_e was significantly larger (n = 18, P < 0.05). The average respiratory rate increased >10% of control in one trial (from 6.2 to 7.0 breaths/min), decreased more than 10% of control in seven trials (from a mean of 7.4 ± 1.1 to a mean of 6.2 ± 1.1), and remained unchanged in ten trials (mean 8.8 ± 0.8). The magnitude of the carbachol-induced suppression was not significantly different between RL-Aug and RLE-Dec activity or between XII-Aug and Phar-E-Aug activity. In contrast, RL-Aug and RLE-Dec activities were significantly (P < 0.05) less suppressed than either XII-Aug or Phar-E-Aug (paired t-tests with Bonferroni correction).

The data are presented in their rank order according to the change of Phar activity after the carbachol injection relative to control (100%). In all but one case, the activities of RL and Phar after carbachol were >60% of control, whereas those of XII and Phar were always <40% of control.

In seven animals, the recovery from the effect of the first, and then second (n = 3), injection of carbachol was produced by pontine injections of atropine. After a new steady state was reached, the nerve activities were in most cases greater than those measured in the precarbachol control state (see Figs. 1 and 2): Phar, eight of ten trials; XII-Aug, eight of nine trials; RL-Aug and RLE-Dec, seven of seven trials; and Phar-E-Aug, six of nine trials. Figure 5 shows the mean amplitudes of the postcarbachol and postatropine activities in each nerve relative to the precarbachol control amplitude (100%). For these 10 trials, the amplitude of Phar activity after carbachol was at 84 ± 4.9% of the precarbachol control (n = 10, P < 0.01), and after atropine it increased to 136 ± 17% of the precarbachol control (n = 10, P < 0.05); for RL-Aug the amplitude was 75 ± 4.8% (n = 7, P < 0.01) and 113 ± 3% (n = 7, P < 0.01), respectively; for RLE-Dec the amplitude was 70 ± 7.3% (n = 6, P < 0.01) and 169 ± 18% (n = 6, P < 0.01), respectively; for XII-Aug, the amplitude was 15 ± 4.4% (n = 9, P < 0.001) and 204 ± 52% (n = 9, P < 0.01), respectively; and for Phar-E-Aug the amplitude was 9 ± 4.1% (n = 10, P < 0.001) and 144 ± 39% (n = 10, P > 0.1), respectively. Thus only the postatropine overshoot of Phar-E-Aug activity did not reach statistical significance.

Carbachol-induced depression of spontaneous cough-like activity. In four animals, short, stereotyped bursts of activity were generated spontaneously in the upper airway nerves at the time of inspiratory offset (Fig. 6; see also Table 1). In an individual animal, the bursts occurred fairly regularly, albeit, among animals, the interburst intervals ranged from 2 to 27 respiratory cycles. The amplitude of the bursts relative to the main respiratory components of activity was always the largest in Phar. Their duration, as measured from Phar activity, ranged from 390 to 520 ms (mean 435 ± 29 ms; n = 4).
In these four animals, seven pontine microinjections of carbachol and five injections of atropine were made. Figure 6 illustrates the results of one experiment (animal 53). In this and one other animal, the cough-like bursts were abolished after carbachol (Fig. 6A), began to appear at full magnitude when signs of a spontaneous recovery could be recognized from the reappearance of a small respiratory modulation in XII and Phar, and occurred with increased frequency after an injection of atropine. In the remaining two animals, the bursts persisted during the maximal suppressant effect of carbachol, but their interburst interval increased to 165 ± 15% of control (4 carbachol microinjections in 2 animals, P < 0.05). After the reversal of the effect of carbachol by pontine atropine, the interburst interval decreased below the precarbachol level to 81 ± 14%, and the amplitude of the bursts in Phar recovered to 101 ± 1.4% of precarbachol control (not significant, P > 0.2, and P > 0.3, respectively, n = 5, paired t-tests). In those two animals (4 microinjections) in which the bursts were not abolished by carbachol, carbachol decreased the mean amplitude of the cough-like bursts in Phar only to 87 ± 2.7% of control (P < 0.05).

Comparison of the effect of carbachol and reduced PICO2 on upper airway nerve activity. As described, carbachol might have produced some depression of the central inspiratory drive, as evidenced by a significant decrease of Phr activity. In inspiratory-modulated up-
per airway motoneurons, which may have steeper input-output characteristics for the central inspiratory drive input than Phr (11), such a depression could contribute importantly to their carbachol-induced decrease in activity. To assess this possibility, we compared the magnitude of the carbachol-induced decrease in upper airway nerve activity to the magnitude of activity that was present when the reduced $P_{CO_2}$

![Graph showing magnitudes of suppression of nerve activities (open bars) and subsequent recoveries from effect of carbachol produced by pontine atropine into same pontine site (hatched bars). Mean magnitudes of Phr, RL$^{E\_Aug}$, RL$^{E\_Dec}$, XII$^{E\_Aug}$, and Phar$^{E\_Aug}$ activities are normalized to their control, precarbachol values. After atropine, activities of all nerves were larger than in precarbachol control, with overshoot being significant in all nerves but Phar. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for comparisons to precarbachol control (paired t-tests); $n = 5$ number of tests with carbachol and subsequent reversals of effect with atropine.]

**Fig. 5.** Magnitudes of suppression of nerve activities (open bars) and subsequent recoveries from effect of carbachol produced by pontine atropine into same pontine site (hatched bars). Mean magnitudes of Phr, RL$^{E\_Aug}$, RL$^{E\_Dec}$, XII$^{E\_Aug}$, and Phar$^{E\_Aug}$ activities are normalized to their control, precarbachol values. After atropine, activities of all nerves were larger than in precarbachol control, with overshoot being significant in all nerves but Phar. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for comparisons to precarbachol control (paired t-tests); $n = 5$ number of tests with carbachol and subsequent reversals of effect with atropine.

![Graph showing effects of carbachol (110 nl) and subsequent atropine (140 nl) microinjections into same site of pontine tegmentum on moving averages of nerve activities in an animal generating coughlike bursts (animal 53). Bursts were abolished after carbachol injection (left arrow) and reappeared after ~8 min when first signs of a spontaneous recovery (small respiratory-modulated activity in Phar, middle arrow) occurred. After a pontine injection of atropine that fully reversed the effect of carbachol (right arrow), bursts occurred even more frequently than in the precarbachol control period. Note that amplitude of coughlike bursts remained constant regardless of carbachol-induced changes in magnitude of respiratory activity in upper airway nerves. B: segments of control (left), and postatropine recovery (right) activity shown on expanded time scale. Additional bursts that, unlike the coughlike bursts, occasionally occurred only in XII were not analyzed.]

**Fig. 6.** A: effect of carbachol (110 nl) and subsequent atropine (140 nl) microinjections into same site of pontine tegmentum on moving averages of nerve activities in an animal generating coughlike bursts (animal 53). Bursts were abolished after carbachol injection (left arrow) and reappeared after ~8 min when first signs of a spontaneous recovery (small respiratory-modulated activity in Phar, middle arrow) occurred. After a pontine injection of atropine that fully reversed the effect of carbachol (right arrow), bursts occurred even more frequently than in the precarbachol control period. Note that amplitude of coughlike bursts remained constant regardless of carbachol-induced changes in magnitude of respiratory activity in upper airway nerves. B: segments of control (left), and postatropine recovery (right) activity shown on expanded time scale. Additional bursts that, unlike the coughlike bursts, occasionally occurred only in XII were not analyzed.
diminished the respiratory drive to the point at which the Phr amplitude was reduced by the same amount as it was by carbachol. The tests with reduced PICO₂ were performed either before the carbachol microinjection or after completion of the protocol with the carbachol and atropine microinjections. Similar results were obtained in all five tests in three animals, and the data were combined.

Figure 7 summarizes the changes in activity induced by carbachol and hypocapnia when the decreases of Phr activity were equivalent (to 3.8% by carbachol and hypocapnia when the decreases of Phr activity were equivalent (to 83% by carbachol and hypocapnia when the decreases of Phr activity was a characteristic of the effect of carbachol because the magnitudes of suppression in these upper airway nerves were similar in all the upper airway nerves. Although we did not directly measure the effect of vagotomy on Phar activity, lung inflation reduces the activity of a wide variety of upper airway nerves and muscles (inspiratory and expiratory; nasal, laryngeal, and pharyngeal) and therefore probably also the activity of Phar (see Ref. 12 for review). In contrast, in nonvagotomized animals or normal human subjects, the activities of XII and pharyngeal motoneurons, or the corresponding muscles, are often variable, making changes in their activity, especially decreases, difficult to quantify.

Under these conditions, we observed a highly consistent and differential suppressant effect of carbachol on upper airway nerve activity. The suppression was always greater in the motor nerves that innervate pharyngeal muscles (Phar and XII) than in either the inspiratory or expiratory components of RL nerve activity. The time courses of the changes in activity induced by carbachol or atropine were similar in all the upper airway nerves, indicating that there is a common system in the pontine tegmentum whose activation by carbachol leads to stereotyped changes in these different upper airway motoneuronal pools.

In the cat, RL innervates the posterior cricoarytenoid (PCA), thyroarytenoid, lateral cricoarytenoid, and ary-
tenoideus muscles. Of those, PCA is a dilator having mostly inspiratory-related, and some expiratory and tonic, activity, whereas the remaining muscles are constrictors with predominantly expiratory activity. Phar innervates the pharyngeal constrictor muscles (cricopharyngeus, thyropharyngeus, and hypopharyngeus), which have mostly expiratory, and some tonic, activity. The respiratory function of these pharyngeal muscles seems to improve airway patency by stiffening the posterior pharyngeal wall (22, 24). Finally, the medial branch of the XII nerve innervates predominantly the genioglossal muscle of the tongue, a pharyngeal dilator with mostly inspiratory, and some early expiratory and tonic, activity (see Refs. 3 and 12 for reviews). The principal distinct components of activity recorded from RL, Phar, and XII nerves closely corresponded to the activities recorded by others from the main upper airway muscles innervated by those nerves. Nevertheless, because we recorded the activity of entire nerve trunks, the interpretation of our results in terms of the behavior of individual upper airway muscles needs to be cautious. For example, RL innervates several muscles that have an E-Dec activity pattern, and the activity of the motoneurons to different muscles may not have been equally suppressed by carbachol. Therefore, the carbachol-induced reduction of RL_E-Dec activity to 72% of control could have resulted from the activity of motoneurons to some muscles being almost completely suppressed and others having very little change. Thus it is possible that the activity of some laryngeal motoneurons contained in the RL was suppressed to a much greater extent than the average for the whole RL_E-Dec activity.

Differential carbachol-induced suppression of upper airway nerve activity. The magnitude of the carbachol-induced suppression was not uniform among the studied pharyngeal and laryngeal nerves. There were minimal differences in the effect of carbachol between RL_1-Aug and RL_E-Dec activity and between Phar_E-Aug and XII_E-Aug activity, with both components of RL activity suppressed much less than the activity in the pharyngeal nerves. Thus the observed differences in the magnitude of the suppression cannot be related to differences between inspiratory- and expiratory-related motoneurons or to the anatomic location of the motoneuronal pools, such as hypoglossal versus ambiguous motor nuclei. In addition, the distinction between laryngeal and pharyngeal motoneurons is also not absolute, because the activity of at least one laryngeal muscle with expiratory activity (arytenoideus) is strongly depressed during REM sleep (20). Thus we could discern no criterion that was helpful in making predictions about the effect of REM sleep on the activity of motoneurons innervating distinct respiratory muscles; rather, each individual muscle seems to have its own characteristic behavior during changes in sleep-wakefulness states.

Not only the magnitude of, but also the mechanisms causing, the reduction of motoneuronal activity during REM sleep (and also carbachol-induced atonia) may be different in different motoneuronal pools. Lumbar motoneurons are subjected to a postsynaptic inhibition (8, 30, 31) that can be abolished by strychnine, a glycnergic antagonist (4). In contrast, neither strychnine nor bicuculline, a GABAergic antagonist, abolishes the carbachol-induced suppression of XII motoneuronal activity (17), and only part of the REM sleep suppression of reflexly evoked trigeminal motoneuronal activity can be explained by an active, postsynaptic inhibition mediated by amino acids (43). Thus it is possible that a combination of mechanisms underlies the suppression of activity in the studied upper airway motoneurons (17, 37).

The observed differences in the magnitude of the suppression must be related to the pattern of innervation of the different motoneuronal pools, the pattern of the effects of pontine carbachol on the activity of the relevant premotor neurons, and the properties of the motoneurons themselves. In particular, carbachol-induced changes in the phasic inspiratory and expiratory drives and in tonic inputs (both excitatory and inhibitory) need to be considered as contributors to the characteristic pattern of the changes in motoneuronal activity. With regard to the tonic inputs, it was previously proposed that a withdrawal of serotonergic excitation plays a major role in the suppression of XII motoneurons during the carbachol-induced atonia (19). In a follow-up study, it was determined that the activity of medullary serotonergic cells with axonal projections to the XII nucleus is depressed during the carbachol-induced atonia (48) and, more recently, it was found that laryngeal motoneurons, both inspiratory and expiratory, are less sensitive than XII motoneurons to the excitatory effects of serotonin (6). Thus laryngeal activity may be less dependent on an excitatory serotonergic drive. This could explain, at least in part, the weaker effect of carbachol on laryngeal than XII motoneurons. In contrast to the tonic serotonergic drive that is withdrawn both in natural REM sleep and the carbachol-induced atonia, central respiratory drive is increased during natural REM sleep (34) and only slightly suppressed in the decerebrate carbachol model (18). Accordingly, we hypothesize that, at least under our experimental conditions, the serotonergic drive in XII, and perhaps also in Phar, motoneurons is more dominant than the respiratory drive, whereas the opposite prevails in laryngeal motoneurons. This difference may contribute importantly to the differential effects of carbachol on pharyngeal and laryngeal motoneurons observed in this study.

Although the reduction in the activity of medullary respiratory neurons elicited by carbachol in the decerebrate cat is slight (18), we had to consider the possibility that changes in central respiratory drive are not uniformly distributed among different pools of upper airway motoneurons, thereby contributing to the observed differences in the magnitude of suppression between pharyngeal and laryngeal activities (see Ref. 12 for review). To assess this, we used an alternative means of decreasing central respiratory drive; we induced a level of hypocapnia sufficient to cause a reduction in Phr activity equal to the reduction caused by
carbachol. At this level of hypocapnia, the suppression of upper airway motoneuronal activity was less than that caused by carbachol for all the analyzed upper airway nerves and, unlike those with carbachol, the magnitudes of suppression were similar (see Fig. 7). Thus, the effects of reducing the central respiratory drive to a level similar to that potentially occurring during the carbachol-induced atonia cannot explain the large differences between laryngeal and pharyngeal activities after carbachol. Although a small contribution of the decreased respiratory drive to the carbachol-induced depression of upper airway nerve activity cannot be excluded, there is a much stronger effect of carbachol exerted through pathways independent of those mediating the respiratory drive.

Relationship to upper airway activity during REM sleep. Pontine carbachol injections in either chronically instrumented, intact, or decerebrate cats produce many signs that are similar to those seen during REM sleep. However, the carbachol model has some important differences: phasic, twitchlike activity rarely occurs, the respiratory rate and pattern is very regular, and the depression of activity in different respiratory motoneuronal groups is exaggerated when compared with that recorded during REM sleep, although the overall pattern is qualitatively similar in the two states (13, 26, 44). With respect to the quantitative differences found in our study, we believe that vagotomy ensured a strong respiratory modulation in all upper airway nerves, which facilitated the assessment of the suppressant effect of carbachol. In REM sleep, a suppression of the activity of pharyngeal muscles is most prominent and consistently seen when the respiratory-related activity is increased, as during experimental hypocapnia (36) or in patients with sleep apnea (22, 28, 29). In contrast, the changes in pharyngeal muscle, including genioglossus, activity that occur during REM sleep under normal conditions in a variety of species are less consistent and often small (23, 39–41). Thus increasing the baseline activity of upper airway motoneurons by various means helps reveal the suppressant effects of sleep on this activity.

Given the large carbachol-induced suppression of the activity of spinal respiratory motoneurons in the decerebrate cat carbachol model compared with natural REM sleep (13), we were surprised to observe a relatively small suppression of RL nerve activity. It was less than that observed in chronically instrumented, intact cats during either natural REM sleep or the carbachol-induced REM sleep-like state (26, 35). In REM sleep, PCA inspiratory activity falls to 75% of waking (control) activity and PCA expiratory activity falls to 30%. During the carbachol-induced, REM sleep-like state, the suppression of PCA inspiratory activity is to 45% of control, and PCA expiratory activity is to 40%. One reason for the difference between those studies in chronic cats and the present study may be the fact that we measured whole nerve activity and not individual muscle activity. Another possible explanation is that in our experiments, due to the vagotomy, the respiratory drive to laryngeal motoneurons was increased. Because this drive undergoes small changes after pontine carbachol (18), the magnitude of the depression of activity expressed relative to the control activity level would be expected to be smaller than in nonvagotomized animals. Thus vagotomy, by increasing the respiratory drive in the studied motoneuronal pools, might have enhanced the contrast between strong effects of carbachol in pharyngeal motoneurons and weak effects in laryngeal motoneurons.

Effects of carbachol-induced atonia on the fictive cough-like activity. In four animals, in addition to the respiratory components of upper airway nerve activity, stereotyped bursts of activity were generated in control conditions. They occurred at the offset of inspiration every few respiratory cycles, suggesting that they were entrained to the respiratory rhythm but generated by a nonrespiratory pattern generator. They were unlikely to represent fictive swallows because the latter occurs at variable points of the respiratory cycle, rather than strictly at the time of inspiratory offset (e.g., Refs. 7 and 33). On the basis of their similarity to the patterns of activity observed by others during fictive cough (e.g., Refs. 7 and 33; see Ref. 46 for review), we refer to those bursts as cough-like although they lack one component typical of cough behavior, augmented inspiratory activity preceding the expiratory burst. Alternatively, these bursts may correspond to fictive sneezes (32).

Regardless of the exact nature of these bursts, they provide additional insight into the changes induced by carbachol in distinct premotor inputs to the studied motoneuronal pools. Carbachol produced two changes in the characteristics of the bursts: a decrease in frequency (in 2 animals, the bursts were abolished) and a decrease of their amplitude in Phar which was still much less than the decrease in the respiratory activity of this nerve. The former indicates that pontine carbachol evokes changes in the neuronal circuits responsible for the initiation of the bursts. These changes were larger than the rate changes produced in the respiratory rhythm generator, providing another example of differential effects of pontine carbachol. Decreases in the burst frequency, up to the point of their abolition, may correspond to the observation that coughs cannot be evoked during natural REM sleep (see Ref. 1 and references therein).

The small carbachol-induced change in the amplitude of the cough-like bursts in Phar contrasts with the profound decrease in the magnitude of the respiratory modulation of Phar activity. Regardless of whether the observed respiratory activity and the cough-like bursts were generated in the same or different Phar motoneurons, this comparison reveals the presence of large differences in the magnitude of the carbachol-induced suppression within the pool of vagal motoneurons innervating a restricted region of the pharynx. This further strengthens the possibility that carbachol, and by extension REM sleep, exerts its effects on different motoneuronal pools and premotor neurons in a highly
selective manner that is specific for different outputs and motor behaviors.

In conclusion, we have used injections of carbachol into the dorsal pontine tegmentum to study the pattern of the REM sleep-like changes in the respiratory motor output to the diaphragm and selected upper airway muscles. We found a stereotyped suppression of activity that was significantly larger in nerves innervating pharyngeal muscles than in nerves to either laryngeal muscles or the diaphragm. Given the similarities between the carbachol-induced atonia and that of REM sleep (2, 25), it is likely that pontine cholinergic neurons affect distinct upper airway motor outputs similarly during both states.

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