μ-Opioid receptor agonist effects on medullary respiratory neurons in the cat: evidence for involvement in certain types of ventilatory disturbances

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OPIOIDS are among the oldest and most frequently used drugs for the alleviation of pain, coughing, and smooth muscle spasticity and are routinely used as anesthetic adjuvants. The major problem in achieving the desired clinical effects of opioids is the cost to breathing. Side effects involving respiration are well known and include depression of tidal volume and gas exchange, slowed rate of breathing progressing to respiratory arrest with higher doses, and blunting of respiratory sensitivity to carbon dioxide (32, 56, 63). In the surgical theater, additional respiratory side effects of opioids are airway obstruction at glottic and supraglottic levels, decreased chest wall compliance, and increased resting tension of abdominal musculature (1, 3, 7, 8, 13, 43, 47, 54). Although these impediments to ventilation are potentially life threatening and occur not infrequently, the sites and mechanisms within the respiratory neural network that give rise to them have not been investigated in detail.

A number of important experimental studies have established that opioids disrupt respiratory rhythm and depress breathing through what appears to be generalized depression of respiratory network activity (31, 32). In support of the concept that opioids exert widespread influence on the bulbospinal respiratory network, studies have shown that endogenous opioids as well as μ- and δ-subtypes of opioid receptors are present in essentially all respiratory regions of the pons and medulla (23, 33, 44, 45, 52, 56, 63). An especially important site of opioid-mediated disturbances of respiratory rhythm may be the pre-Bötzinger complex, a region of the medulla thought to be essential for generation of a normal respiratory rhythm (57) and where exogenous opioids modulate rhythm and hyperpolarize inspiratory neurons (23, 49). Investigations performed on intact animals and on medullary slice and brain stem-spinal cord preparations of neonatal rodents have revealed that opioids have presynaptic and postsynaptic effects that alter excitability of bulbospinal respiratory neurons (6, 15, 22–24, 26, 33, 44, 49, 55, 59).

There are relatively few published reports that can implicate specific types of bulbospinal respiratory neurons in particular kinds of opioid-mediated ventilatory disturbances (24, 29, 41, 45, 62).
In the present study, the dose-related effects of the μ-opioid receptor agonist fentanyl given systemically on membrane and discharge properties of medullary respiratory neurons in adult cats were measured. Axonal projections of medullary respiratory neurons were also determined electrophysiologically, allowing them by virtue of destination to be implicated in the control of tidal volume, chest wall compliance, or upper airway flow (10). In addition, the effects of the μ-opioid receptor pentapeptide agonist DAMGO administered by iontophoresis on discharge properties of medullary neurons were recorded to further test whether μ-opioid receptor-mediated effects were activated postsynaptically. To determine how anesthesia influences the occurrence of μ-opioid receptor-mediated disturbances, experiments were performed on both pentobarbital-anesthetized and unanesthetized midcollicular decerebrate cats.

The goals of this study were 1) to analyze opioid-mediated neural mechanisms that can account for the depression of tidal volume, decreased chest wall compliance, and increased airway resistance seen clinically; 2) to determine how the opioid-mediated disturbances develop as a function of dose; and 3) to find out whether general anesthesia is obligatory for the respiratory network disturbances.

Preliminary accounts of this work have been published in abstract form (37, 38).

MATERIALS AND METHODS

Animal preparation. Data described in this study were obtained from experiments performed on 67 adult cats in the Department of Physiology, University of Wisconsin. Fifty-five of the animals were pentobarbital-anesthetized cats, and another 12 were unanesthetized decerebrate preparations. Animals of either sex weighing 3.0–5.7 kg were used. Care and use of animals were in accordance with the guiding principles of the University of Wisconsin Animal Care Committee and the American Physiological Society. In 55 experiments, anesthesia was induced with halothane in a chamber (5% halothane in oxygen, 5 l/min gas flow) followed by administration through a mask (2.5% halothane in oxygen, 5 l/min gas flow) by adjusting ventilatory rate and tidal volume. Temperature was measured rectally and maintained at 36–38°C by external heating. Pneumothorax was performed bilaterally to increase stability of recording from medullary respiratory neurons. Applying 1- to 2-cmH2O pressure to the expiratory outflow prevented atelectasis. Animals were single-craniotomied and monted in a stereotaxic head holder and suspended by thoracic and lumbar spinal clamps. Phrenic nerves (C1–C2) and cervical vagus nerves were exposed bilaterally through a dorsal approach and sectioned; their central ends were desheathed, and the nerve trunks were mounted on bipolar silver hook electrodes and covered with a mixture of Vaseline and mineral oil. The head of the animal was ventroflexed to allow wide exposure of the dorsal surface of the medulla by occipital craniotomy. The dura and arachnoid membranes were reflected, and patches of pia membrane were removed to allow insertion of fine-tipped glass microelectrodes. A pressure foot was placed gently on the surface of the medulla over the site of microelectrode insertion. A cerebral laminectomy (C3–C4) was performed, and the dura was cut and reflected for insertion of stimulating electrodes. After placement of electrodes, the spinal cord and other exposed tissue except for the medulla was covered with warm (37°C) Ringer solution containing 3% agar.

Recording procedures and measurements. Phrenic nerve activity was amplified (2,000–10,000×; Grass Instruments, Quincy, MA), band-pass filtered (100–3,000 Hz), displayed on an oscilloscope (Tektronix Instruments, Beaverton, OR), and registered on magnetic tape (frequency response, DC-5 kHz; Vetrone Technology, Rebersburg, PA) and on chart recorder paper (DC-10 KHz; Gould, Cleveland, OH) as raw discharges (neurograms) and as moving averages (τ = 100 ms) of compound action potential frequency. To obtain quantitative estimates of phrenic nerve activity, measurements included 1) the peak frequency of compound action potentials obtained from the moving average, 2) the durations of the inspiratory, postinspiratory, and expiratory phases of the cycle, 3) the frequency of inspiratory phase bursts of compound action potentials, and 4) the rate at which compound action potential frequency increased to reach a peak (spike frequency augmentation), a value obtained from the slope of the moving average curve.

Intracellular recordings were obtained with fine-tipped glass micropipettes filled with 2 M K-methylsulfate. DC resistances of the microelectrodes ranged from 50 to 80 MΩ. Membrane potentials were recorded in bridge or discontinuous single-electrode current-clamp mode with amplifiers for intracellular recording (Bandwidth, DC-10, 000 Hz; SEC 05; npi, Tamm, Germany or Dagan 8500, St. Paul, MN). Neuron input resistance measurements were made by injecting 60- or 80-ms negative-going constant-current pulses through the microelectrode in current-clamp mode and measuring the
resulting hyperpolarizing voltage drop across the cell membrane. Electrophysiological data were also acquired and stored on computer and CD with PowerLab hardware and software (AD Instruments, Castle Hill, NSW, Australia). Voltage-clamp measurement of membrane currents was made in one experiment during discontinuous single-electrode voltage clamp (switching frequency 30 kHz, 25% duty cycle). The procedures for voltage clamping respiratory neurons in vivo were previously described in detail (40).

Identification of bulbospinal respiratory neurons and vagal motoneurons. To identify bulbospinal inspiratory and expiratory neurons, two concentric coaxial electrodes (SNEX-100, A-M Systems, Everett, WA) were positioned bilaterally in the cervical reticulospinal tracts at the C3 level. Stimulation with single shocks applied bilaterally (5 shocks were also sufficient) with different ejecting currents, each for at least 2 min. The procedures for voltage clamping respiratory neurons were retained with 5-nA anionic current and ejected with NaCl from the current-balancing micropipette. Chemicals used in this study included: naloxone (RBI) 10 mM; naltrindole (RBI), 10 mM; and naloxonazine (RBI) 20 mM, in aqueous solution; and the iontophoresis barrels with drug solutions and current-balancing electrolyte.

Neuron discharges were recorded at unity gain in bridge recording mode (DAGAN 8500, St. Paul, MN), amplified 1,000×, and band-pass-filtered at 100–3,000 Hz with an AC preamplifier (Grass P511), monitored on the oscilloscope, and recorded as neurograms and moving averages on the chart recorder. Chemicals for iontophoretic application were dissolved in bidistilled water and adjusted to pH 4.5 with 0.1 N HCl. Iontophoresis barrels contained 1) the selective μ-opioid receptor agonist [d-Ala²,N-Me-Phe³,Gly-ol⁴]enkephalin [DAMGO, Research Biochemicals International (RBI), Natick MA], 10 mM in aqueous solution; 2) the μ-opioid receptor blocker naloxonazine (RBI) 10 mM; 3) the δ-opioid receptor blocker naltrindole (RBI), 10 mM; and 4) NaCl, 165 mM, for iontophoresis current balancing. Control tests for current-evoked effects were also made by iontophoresis of 165 mM NaCl from the current-balancing micropipette. Chemicals were retained with 5-nA anionic current and ejected with 50- to 90-nA cationic currents by a programmable iontophoresis current generator (Dagan 6400). The iontophoresis protocol involved application of DAMGO at least twice with different ejecting currents, each for at least 2 min. Full recovery was allowed after each application. After DAMGO had produced significant depression of neuronal discharges in at least two test runs, an opioid receptor blocker was iontophoresed 3 min before and concurrently with DAMGO for several minutes. After recovery, DAMGO alone was reapplied until the opioid depressant effects had returned to original levels.

Intravenous administration of fentanyl and naloxonazine. The μ-opioid receptor agonist fentanyl citrate (Sigma Chemical; 0.04 or 0.4 mg/ml in Ringer solution) or naloxonazine (0.2 mg/ml in Ringer) was injected slowly over a period of ~30 s to minimize changes of blood pressure and avoid loss of stable microelectrode recording. Fentanyl was administered in increments of 1 μg/kg to detect threshold disturbances of phrenic nerve activity and membrane potential in medullary respiratory neurons, and then doses of 2–5 μg/kg were given to follow the dose-related progression of disturbances until phrenic nerve apnea was induced. In some tests made on caudal expiratory neurons and laryngeal postinspiratory neurons, additional increments of 10–20 μg/kg were tested after phrenic nerve apnea had occurred.

Euthanasia. Experiments were terminated by intravenous injection of pentobarbital sodium in sufficient quantity to produce irreversible cardiac arrest.

Statistical analysis. SigmaPlot version 4.11 software (Jandel Scientific) was used to obtain the means and SEs of pooled data and for paired T-tests to determine significance of difference. Differences were accepted as significant if P < 0.05.

RESULTS

Effects of systemically administered fentanyl on phrenic nerve discharge properties. Rhythmic discharges of the phrenic nerve reflect the integrative behavior of the brain stem respiratory network and its motor output to the diaphragm. To determine how μ-opioid receptor agonists affect respiratory network rhythm and discharge intensity as a function of dose, the effects of graded doses of the selective μ-opioid agonist fentanyl on phrenic nerve discharges were measured. Dose increments were given as described above (see MATERIALS AND METHODS) every 2–4 min until a cumulative dose, i.e., the sum of dose increments, abolished phrenic nerve discharges (central apnea).

In both anesthetized and decerebrate cats, phrenic nerve activity under control conditions exhibited a prototypical three-phase cycle, consisting of an augmenting discharge of compound action potentials during the inspiratory phase, a subsequent discharge with declining action potential frequency during the postinspiratory phase, and, last, a silent period (expiratory phase) as described by others (10, 50). The cycle was repeated at a relatively steady frequency in the absence of drug intervention (range, 11–21/min for all experiments). Examples of the rhythm are illustrated in the control traces of Fig. 1.

Fentanyl disturbed the respiratory rhythm and depressed discharge intensity without abolishing action potentials in doses ranging from 3 to 20 μg/kg. The effects of subapneic doses of fentanyl, which appeared 20–30 s after each effective dose, are summarized in Table 1 and illustrated in records of Fig. 1.

Threshold doses of 3–5 μg/kg significantly (P < 0.05) prolonged inspiratory (T₁) and expiratory phase durations (Tₑ), and consequently burst frequency (F_b) was reduced. Spike frequency augmentation (∆Fₛ/∆T) was also significantly reduced. Threshold doses did not significantly change the duration of the postinspiratory phase (Tₚₑ) among experiments (ablation of Tₚₑ after a
10 μg/kg dose in Fig. 1 was 1 of 2 exceptions) or the peak compound action potential frequency (PFs).

Cumulative doses of 7.5–20 μg/kg significantly depressed PFs, further prolonged TEx, and further reduced FB and ΔFs/ΔT. Further prolongation of TI did not occur, and TPI did not change significantly. Apnea occurred when cumulative doses reached 23 ± 3.2 μg/kg.

In unanesthetized decerebrate cats, threshold doses were the same (3–5 μg/kg), and dose-related disturbances were similar to those in anesthetized preparations, except that the average prolongation of TI was significantly less (by 220 ± 19 ms, n = 10 cats, P < 0.05). To produce apnea, a mean dose of 32 ± 3.4 μg/kg was required. This dose was significantly greater (P < 0.05) than the apneic dose in anesthetized cats.

During apnea, phrenic nerve activity was either totally absent or consisted of uninterrupted low-amplitude, low-frequency compound action potentials or brief, sporadic low-intensity bursts (see Figs. 3C and 8A2). Phrenic nerve discharges returned to their original intensity and rhythm within 45–56 min after the onset of apnea.

Within the range of 13–20 μg/kg given in increments, fentanyl had no significant effects on blood pressure or heart rate in anesthetized or decerebrate cats.

Tests of μ-opioid receptor agonists on membrane properties and discharges of medullary respiratory neurons. Neurons that play key roles in the control of tidal volume and upper airway resistance were sought to determine how they are affected by opioids given sys-

Table 1. Disturbances of phrenic nerve discharge rhythm after subapneic intravenous doses of fentanyl

<table>
<thead>
<tr>
<th>Dose Range</th>
<th>TI (s)</th>
<th>TPI (s)</th>
<th>TEx (s)</th>
<th>FB (min)</th>
<th>PFs (spikes/s)</th>
<th>ΔFs/ΔT (spikes/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n = 27</td>
<td>1.42 ± 0.07</td>
<td>0.95 ± 0.09</td>
<td>1.65 ± 0.12</td>
<td>15.9 ± 0.72</td>
<td>796 ± 23.3</td>
<td>1,693 ± 103.8</td>
</tr>
<tr>
<td>Fentanyl, 3–5 μg/kg (mean 4.3 ± 0.16 μg/kg), n = 27</td>
<td>2.70 ± 0.28*</td>
<td>1.40 ± 0.14</td>
<td>2.91 ± 0.30*</td>
<td>10.0 ± 0.8*</td>
<td>816 ± 19.3</td>
<td>1,516 ± 97.8*</td>
</tr>
<tr>
<td>Fentanyl, 7.5–20 μg/kg (mean 13.4 ± 1.8 μg/kg), n = 15</td>
<td>2.53 ± 0.23*</td>
<td>1.01 ± 0.20</td>
<td>3.97 ± 1.42†</td>
<td>9.1 ± 0.80*</td>
<td>569 ± 75.1†</td>
<td>469 ± 135.9†</td>
</tr>
</tbody>
</table>

Values are means ± SE. TI, duration (s) of inspiratory phase; TPI, duration (s) of postinspiratory phase; TEx, duration (s) of expiratory phase; FB, frequency of phrenic nerve discharges (bursts/min); PFs, peak frequency of phrenic nerve compound action potentials (spikes/s); ΔFs/ΔT, rate of increase in spike frequency during the inspiratory phase. *Significantly different from control; †Significantly different from control and from effects of lower dose range (3–5 μg/kg) of fentanyl.

R1290 OPIOID DISTURBANCES IN BULBAR RESPIRATORY NEURONS

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OPIOID DISTURBANCES IN BULBAR RESPIRATORY NEURONS

Effects of systemically administered fentanyl on membrane properties of inspiratory neurons. The effects of intravenously administered fentanyl on membrane potential and neuron input resistance were measured in three different types of medullary inspiratory neurons: 1) bulbospinal Aug-I neurons (n = 21; 6 from decerebrate cats), which have been shown to provide excitatory synaptic drive to phrenic and inspiratory intercostal motoneurons (10, 19, 42) and thus regulate tidal volume; 2) inspiratory neurons that exhibited constant-amplitude, plateau-like depolarizing synaptic drive potentials (Con-I neurons, n = 6, all from anesthetized cats) and were not antidromically activated by spinal cord or vagus nerve stimulation but which might have synaptically activated bulbospinal inspiratory neurons (18); and 3) vagal Aug-I motoneurons (n = 8, 3 from decerebrate cats), which are reported to control the cricothyroid (abductor) muscles of the vocal folds (4, 10). The bulbospinal neurons were located 1–3 mm rostral to the obex, 3.4–3.8 mm lateral to the midline, and 3.2–4.0 mm below the dorsal surface. Vagal motoneurons were located 1–2 mm rostral, 3.4–3.8 mm lateral, and 3.4–3.6 mm ventral. Con-I neurons were 3.0–3.6 mm rostral, 3.6–3.8 mm lateral, and 3.4–4.0 mm ventral. The cellular responses to fentanyl were similar in decerebrate and anesthetized cats.

Bulbospinal Aug-I neurons. Figures 2 and 3 show the effects of fentanyl on bulbospinal Aug-I neurons (n = 21). In all of the neurons tested, fentanyl in lowest effective doses (3–5 μg/kg) slowed rhythm without affecting firing threshold, peak action potential frequency, or spike afterhyperpolarization (Fig. 2). Inspiratory phase depolarizing synaptic drive potentials (SDPs) and discharges were prolonged, and the rates of depolarization and spike augmentation were slowed. In addition, membrane potential was hyperpolarized during the expiratory silent period, which was also prolonged. Larger subapneic doses of fentanyl (5–17 μg/kg) reduced the amplitude of depolarizing SDPs and further slowed the rate of depolarization.

As shown in Fig. 3, cumulative doses of 20 μg/kg or more hyperpolarized membrane potential, abolished SDPs, and eliminated action potentials in parallel with...
abolition of phrenic nerve activity. In association with membrane hyperpolarization, neuron input resistance decreased (e.g., Fig. 3C) from 8.3 ± 1.4 to 4.4 ± 0.8 MΩ (P < 0.05). In tests on 10 bulbospinal neurons, depolarization with steady cationic current caused the neurons to fire, but values of input resistance were not significantly different from those that accompanied opioid-induced membrane hyperpolarization. After administering the μ-opioid receptor antagonist naloxonazine (25–100 μg/kg), rhythmic membrane potential oscillations and discharges along with phrenic nerve activity were not only reinstated, they were more intense and of higher frequency than control levels (Fig. 3D).

Iontophoresis of the μ-opioid receptor agonist DAMGO onto bulbospinal inspiratory neurons. Because reduction of neuron input resistance and hyperpolarization of membrane potential suggest postsynaptic inhibition, this possibility was further tested by applying the μ-opioid receptor agonist DAMGO iontophotically to bulbospinal Aug-I neurons during extracellular recording in 15 pentobarbital-anesthetized cats. This agonist was chosen because DAMGO is not only selective for μ-opioid receptors, it is inactivated by membrane-bound and extracellular enkephalinases (12). Thus the likelihood of opioid spread and its consequent effects on presynaptic neurons during iontophoresis should be minimal.

DAMGO was tested on 25 antidromically activated bulbospinal Aug-I neurons of the VRG located 1–3 mm rostral to the obex, 3.4–3.8 mm lateral to the midline, and 3.2–4.0 mm below the dorsal surface. Under control conditions, the neurons exhibited augmenting bursts of action potentials that coincided with or shortly followed the onset of PNA discharges (Fig. 4). Iontophoretic application of DAMGO with 50- to 90-nA ejecting currents reduced action potential frequency of all Aug-I bulbospinal neurons within 1–3 min of application without affecting phrenic nerve activity. The longer latency to effect compared with that
of fentanyl given intravenously was probably due to factors associated with iontophoretic delivery of drugs (37), including the time required to recharge the drug concentration at the microelectrode tip after back-diffusion caused by retaining current and for its diffusion across brain tissue barriers and buildup to effective concentrations at opioid receptors. The onset of discharges with respect to PNA was delayed by DAMGO iontophoresis; consequently burst durations were shortened (Fig. 4B2). Control activity was restored within 60–90 s in the 25 bulbospinal neurons after terminating iontophoresis.

Concurrent iontophoresis of the μ-opioid receptor blocker naloxonazine (50–90 nA) diminished the depressant effects of DAMGO on 15 bulbospinal VRG neurons tested (e.g., Fig. 4B3). In the presence of naloxonazine, DAMGO-mediated depression of peak action potential frequency was reduced from 50–85% to 20–35%. The δ-opioid receptor blocker naltrexone (50–100 nA) in tests on 10 of the 15 cells failed to antagonize the effects of DAMGO.

Con-I propriobulbar neurons. The dose-related effects of intravenously administered fentanyl on Con-I neurons (n = 6) were identical to those on bulbospinal Aug-I neurons (Fig. 5). Threshold doses (3–5 μg/kg) of fentanyl slowed rhythm without changing peak action potential frequency. Depolarizing SDPs were prolonged and depressed by larger subapneic doses of fentanyl that also depressed and slowed phrenic nerve activity. Cumulative doses of 20 μg/kg or more abolished rhythm in association with 1) a steady level of membrane hyperpolarization (5.5 ± 0.65 mV more...
decreased neuron input resistance (from 5.7 \pm 1.2 \Omega during the control expiratory silent period to 2.7 \pm 0.5 \Omega, \ P < 0.5); and 3) phrenic nerve apnea.

Vagal Aug-I motoneurons. In common with effects on Aug-I bulbospinal and Con-I propriobulbar neurons, threshold doses of fentanyl (3–5 \mu g/kg) slowed rhythm and prolonged discharges of vagal Aug-I motoneurons (n = 8) without affecting peak action potential frequency (Fig. 6, A and B). Doses of fentanyl that abolished phrenic nerve activity also abolished SDPs. Membrane potential did not hyperpolarize; rather it stabilized at a steady level that was about midway between the peaks of the control depolarizing and hyperpolarizing SDPs. Action potentials discharged continuously but at very low frequency (9.3 \pm 2.6\% of control spike frequency). A second notable difference between vagal Aug-I neurons and the other two types of inspiratory neuron was that input resistance did not change (not illustrated), suggesting that the opioid disturbance was evoked presynaptically.

Further evidence that opioids do not directly depress the neurons was obtained in three iontophoresis experiments. Direct application of DAMGO with 25- to 90-nA ejecting currents onto 10 vagal Aug-I motoneurons recorded extracellularly failed to alter discharge properties.

Aug-E neurons of the caudal medulla. Expiratory bulbospinal neurons of the caudal medulla provide excitatory synaptic drive to intercostal interneurons and motoneurons (34) as well as to abdominal motoneurons and through them control compliance of the chest wall (10, 42). Opioids have been reported to evoke prolonged extracellularly recorded discharges of caudal medullary Aug-E neurons (41) and expiratory intercostal motor nerve fibers (29), leading to tonic firing as dose is increased, and when administered in even larger doses to abolish firing of Aug-E neurons (41). To examine the cellular mechanisms responsible for the dose-dependent alterations of discharge, intracellular recordings were obtained from 34 bulbospinal expiratory neurons with augmenting depolarizing SDPs and discharges that occurred late in the expiratory phase (Aug-E neurons). Eight of the neurons were recorded from unanesthetized decerebrate cats. Responses were also recorded in anesthetized cats from seven additional Aug-E neurons that were not antidromically activated but exhibited excitatory postsynaptic potentials (EPSPs), inhibitory postsynaptic potentials (IPSPs), or IPSP-EPSP combinations in response to spinal cord stimulation. They were not antidromically activated by vagus nerve stimulation and are referred to hereafter as propriobulbar Aug-E neurons. All of the 41 Aug-E neurons were recorded 1–2 mm caudal to the obex, 2.5–3.5 mm lateral to the midline, and 2.0–2.7 mm ventral.

The effects of fentanyl on membrane properties of the Aug-E neurons are illustrated in Figs. 7 and 8. There were no differences between bulbospinal and propriobulbar Aug-E neurons or between anesthetized and decerebrate preparations in the dose-related responses to fentanyl.

Threshold doses (3–5 \mu g/kg) slowed the rate of membrane depolarization to firing threshold, reduced the amplitude of hyperpolarizing SDPs, and prolonged their duration in parallel with lengthening of inspiratory phase phrenic nerve discharges. The durations of depolarizing SDPS and thus action potential discharges were prolonged, but peak depolarizing SPD amplitude and peak action potential frequency were unchanged (Fig. 7).

Fig. 5. Fentanyl given intravenously slows rhythm and depresses excitability in dose-dependent fashion in non-antidromically activated Con-I inspiratory neurons of the rostral medulla of a pentobarbital-anesthetized cat. Panels illustrate effects of intravenous fentanyl on a propriobulbar inspiratory neuron with constant amplitude depolarizing synaptic drive potentials and action potential discharge patterns. Traces (top to bottom) in each panel are membrane potential, the moving average of phrenic nerve action potential frequency, and the phrenic nerve electrogram. Regularly spaced downward deflections of membrane potential were produced by 1-nA, 60-ms constant-current hyperpolarizing pulses applied for the purpose of measuring neuron input resistance. Values of input resistance (mean of 10 consecutive measurements \pm SE) are presented for control recording and after a total fentanyl dose of 20 \mu g/kg.
Larger doses of 5–15 μg/kg depressed depolarizing SDPs and further increased their duration and rate of depolarization to threshold. The discharges were consequently even more prolonged, while peak action frequency was reduced. Hyperpolarizing SDPs were further reduced in amplitude and, in conjunction with more prolonged phrenic nerve discharges, were further lengthened.

Increasing the cumulative dose to 20–40 μg/kg in 21 bulbospinal neurons and 4 propriobulbar neurons abolished both excitatory and inhibitory SDPs. Membrane potential, which under control conditions was $-52 \pm 3.6$ mV in the late expiratory phase and $-65 \pm 2.8$ mV during the inspiratory phase ($n = 16$ cells), became steady after drug administration at $-60 \pm 3$ mV. Tonic firing occurred at a lower frequency (15–20 spikes/s) than control (20–40 spikes/s). Such disturbances of rhythm and excitability evoked in a bulbospinal Aug-E neuron are illustrated in Fig. 8A2. In this example, tonic firing of the Aug-E neuron was arrested briefly by membrane potential hyperpolarization in association with short, sporadic low-amplitude bursts of phrenic nerve activity. During tests on six Aug-E neurons, hypercapnea (52 mmHg CO2) was produced by intermittent ventilation with a mixture of 5% CO2-95% O2. This treatment restored respiratory-related rhythm and discharge within 90–180 s. Tonic nonrhythmic discharges returned to their original states within 4 min after a return to the original O2-enriched gas mixture.

Cumulative doses of 60 μg/kg ($n = 6$ bulbospinal neurons, 3 propriobulbar neurons) abolished action potential discharges and hyperpolarized membrane potential to steady levels that were $5.4 \pm 1.3$ mV more negative than the control spontaneous inspiratory phase membrane hyperpolarization (Fig. 8B2).

None of the dose-related changes altered neuron input resistance significantly in any of the Aug-E neurons. After the largest doses that abolished firing and hyperpolarized membrane potential, there was a small

Fig. 6. Fentanyl given intravenously slows the rhythm of vagal Aug-I motoneurons in threshold doses and depresses excitability in higher doses in a decerebrate unanesthetized cat. Traces in A–C are membrane potential and electrogams of phrenic nerve activity. After 10 μg/kg, inspiratory phase discharges are prolonged and membrane hyperpolarization is less. After a cumulative (total) dose of 30 μg/kg, depolarizing and hyperpolarizing synaptic drive potentials were abolished and discharge intensity was greatly reduced. Neuron input resistance (not illustrated) was unchanged.
increase in input resistance, from $8.4 \pm 0.8$ to $10.2 \pm 1.2$ MΩ ($P > 0.05$). In five bulbospinal Aug-E neurons, membrane depolarization with applied DC current during fentanyl-induced membrane hyperpolarization evoked tonic firing, and neuron input resistance was reduced but not significantly to $7.8 \pm 0.6$ MΩ ($P > 0.05$).

It was not possible in most of the cells to maintain recording stability until spontaneous recovery occurred. In four cells, however, return of rhythmic Aug-E activity to control after apneic doses occurred 45–56 min after the last dose and recovered in parallel with phrenic nerve activity. In two other Aug-E neurons, naloxonazine (50 µg/kg) administered during apnea evoked by 60 µg/kg fentanyl reinstated discharges along with phrenic nerve activity to levels that exceeded control.

**Vagal postinspiratory neurons.** Vagal motoneurons that depolarize with the onset of phrenic nerve postinspiratory activity innervate the adductor muscles of the larynx (10). In rats, there is evidence that opioid receptor agonists injected into the blood flow to the right cardiac atrium act on peripheral vagus sensory nerve receptors to induce tonic recurrent laryngeal nerve discharges and to increase laryngeal airway resistance (62). To test for effects occurring at central sites of action in the present study, measurements of the effects of fentanyl given intravenously were made on 10 postinspiratory neurons that were antidromically activated by vagus nerve stimulation (e.g., see Fig. 10B) in bilaterally vagotomized cats.

Under control conditions, the motoneurons rapidly depolarized and began discharging during the postinspiratory phase of phrenic nerve activity (Figs. 9A and 10A). The firing onset of the neuron illustrated in Fig. 10 was variable at the beginning of recording (Fig. 10A) but after fentanyl occurred con-
sistently near the onset of postinspiratory phase membrane depolarization.

The dose-related effects of fentanyl were similar to those recorded from caudal Aug-E neurons. Threshold doses (3–5 μg/kg) prolonged the bursts of action potentials (Figs. 9B and 10C). Cumulative doses of 10–15 μg/kg that severely reduced phrenic nerve activity correspondingly depressed inspiratory phase-related hyperpolarizing SDPs so that firing became continuous (Fig. 9C). Doses greater than 20 μg/kg brought membrane potential to steady levels that were more negative by 3.5 ± 1.6 mV than inspiratory phase membrane hyperpolarization under control conditions, a change that was statistically significant, and eliminated neuron discharges (Fig. 10E). All of the dose-related effects occurred without significant changes of neuron input resistance.

In one experiment, iontophoretic administration of DAMGO, 30–90 nA, to four vagal postinspiratory neurons recorded extracellularly was without effect on discharge properties.

Aug-E neurons of the rostral medulla. Late discharging inspiratory neurons with augmenting patterns (n = 4) were found in the same region where vagal inspiratory and postinspiratory motoneurons were located. The neurons were not activated antidromically by stimulation of the cervical trunk of the vagus nerve. They were most likely pharyngeal constrictor motoneurons (10), which have cell bodies in nucleus ambiguous (14) where vagal respiratory motoneurons are located and are not activated by stimulation of the main trunk of the cervical vagus nerve because they send axons to the pharyngeal musculature in the pharyngeal nerve branch (64). Rostral Aug-E neurons were located 2 mm rostral to the obex, 3.5–3.6 mm lateral to the midline, and 3.4–3.6 mm ventral. Under control conditions, they exhibited membrane properties that were similar in pattern and timing to caudal Aug-E neurons.

In common with the dose-related changes evoked in all types of respiratory neurons analyzed, threshold doses (3–5 μg/kg) slowed rhythm and prolonged discharges without affecting peak action potential frequency, whereas larger doses (5–15 μg/kg) further prolonged inspiratory and expiratory phases. The rate of membrane depolarization to threshold was slowed, depolarizing synaptic drive potentials were reduced in amplitude (from 10 ± 2.4 to 5 ± 1.1 mV), and action potential frequency was reduced (from 44 ± 14.4 to 21 ± 5.6 s⁻¹).

In one of the neurons, membrane currents and membrane potential were recorded, as shown in Fig. 11. Under control conditions, the neuron exhibited a prom-
inent barrage of IPSPs during inspiratory phase membrane hyperpolarization (Fig. 11A) and inhibitory postsynaptic currents (IPSCs) accompanying an outward wave of current (Fig. 11B). In late expiration, depolarizing synaptic drive potentials gave rise to action potential discharges, and bursts of excitatory postsynaptic currents (EPSCs) occurred at the peak of the inward wave of current.

A cumulative 25 μg/kg dose of fentanyl greatly reduced phrenic nerve activity and abolished inspiratory phase IPSPs and IPSCs and expiratory phase excitatory drive potentials and currents. Measurements of membrane potential in the three other rostral Aug-E neurons also revealed depression of inhibitory synaptic noise, depolarizing drive potentials, and action potential frequency. Accompanying these changes was an increase of input resistance, from 8 ± 2.8 to 14 ± 4.6 MΩ.

Doses of fentanyl >30 μg/kg abolished depolarizing SDPs along with phrenic nerve activity. Membrane potential stabilized at a level that was about midway between the peaks of the control depolarizing and hyperpolarizing SDPs. Action potentials discharged continuously at very low frequency (8 ± 2.6 s⁻¹).

DISCUSSION

The primary goal of this study was to investigate opioid-mediated neural mechanisms responsible for depression of tidal volume, decreased chest wall compliance, and reduced airway patency. Control of tidal volume, chest wall compliance, and laryngeal and supraglottic airway resistance involves neuronal mechanisms that are relatively well understood. The neurons studied were linked to their respective ventilatory functions from axonal projections and by rhythms and discharge patterns, and new information about the effects of opioids on membrane properties is presented. The linkage of opioid-mediated changes of neuron behavior to specific disturbances of ventilation based on known anatomic and functional properties of the neurons (10, 42) is implied because it was not possible to make measurements of mechanical disturbances evoked by opioids.

A second goal was to determine how the opioid-mediated disturbances develop as a function of dose. The importance of this information is that it establishes whether the doses are within the range used in human and veterinary medical procedures.
sponse analysis also sheds light on whether the disturbances occur through primary effects on the rhythm-generating components or on those that provide tonic drive to the brain stem respiratory network.

The third objective was to find out whether general anesthesia is obligatory for the disturbances. This is an important issue because opioids are commonly used in combination with general anesthetics and other types of central nervous system depressants, for example with barbiturates and benzodiazepines, which also cause respiratory depression. Comparisons between anesthetized and unanesthetized decerebrate animals were therefore important.

In the paragraphs that follow, data related to the study objectives and their functional implications are discussed. Figure 12 shows where \( \mu \)-opioid receptor agonists may be acting to affect the respiratory disturbances addressed in this study.

Fig. 10. Dose-related effects of intravenous fentanyl in a vagal postinspiratory motoneuron in a pentobarbital-anesthetized cat. Apneic doses hyperpolarize membrane potential and abolish firing without significant change of input resistance. Traces in A and C are membrane potential, the moving average of phrenic nerve action potential frequency (fPNA), and the phrenic nerve electrogram (PNA). A: control records. C: after 5 \( \mu \)g/kg fentanyl. Neuron discharge is prolonged in parallel with prolongation of the phrenic nerve postinspiratory and expiratory phases. E: membrane potential hyperpolarization and abolition of neuron discharge (top trace) and phrenic nerve apnea (middle and bottom traces) after a cumulative 40 \( \mu \)g/kg dose of fentanyl. D: 2 traces showing MP with regularly spaced hyperpolarizing potential evoked by 1 nA, 60-ms constant-current pulses, under control conditions (D1) and after 40 \( \mu \)g/kg fentanyl (D2). Action potentials are blanked out in D1. Input resistance was not significantly changed during membrane potential hyperpolarization. B: all-or-nothing antidromic response after membrane potential was hyperpolarized by 40 \( \mu \)g/kg fentanyl. The central end of the vagus nerve was stimulated with single shocks at just-threshold pulse intensity and duration (1.9 V, 100 ms).

Dose-related effects of fentanyl and their clinical relevance. Most of the respiratory network disturbances analyzed in this study occurred with subapneic doses of fentanyl that are within the therapeutic range for augmenting anesthesia and producing analgesia in humans (30). All clinical doses of opioids produce varying degrees of respiratory depression that normally have no observable effect on ventilation but are reflected in higher levels of alveolar and arterial \( P_{\text{CO}_2} \) (32). Therapeutic doses of opioids for the alleviation of pain can, however, produce obvious depression of ventilation in the elderly and in patients with cardiopulmonary disease in whom chronic hypercapnea leads to desensitization of the respiratory network to \( \text{CO}_2 \) and in individuals with central sleep apnea and after cerebrovascular accidents (56). Respiratory rhythm is most sensitive to disturbance by systemic administration of \( \mu \)-opioid receptor ago-

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nists such as fentanyl. Based on reports from other laboratories (17, 48, 58), it was recently pointed out (46) that declines in the magnitude of inspiratory activity are indicative of suppressed tonic drive, whereas selective disruption of rhythm implies that the rhythm-generating neural apparatus is affected. Threshold doses of fentanyl given intravenously in the present study altered rhythm. They prolonged phrenic nerve inspiratory bursts and discharges in all of the different types of respiratory neurons tested and lengthened the interburst silent periods without affecting peak action potential frequency. This finding suggests that neurons that control phase switching and thus rhythm in the bulbar respiratory network (10, 18, 20, 50, 51) are more sensitive to \( \mu \)-opioid receptor agonists than those that supply tonic drive to respiratory neurons. A potential site of opioid action might be respiratory neurons of the pre-Bötzinger complex.

The prolongation of discharge and reduced rate of action potential augmentation produced in bulbospinal Aug-I neurons and phrenic nerve activity by intravenous fentanyl, although not a central issue in this study, were consistent and noteworthy. Discharge prolongation is likely to be related to presynaptic effects, perhaps on inspiratory off-switch neurons (50) because iontophoresis of DAMGO onto bulbospinal Aug-I inspiratory neurons had the opposite effect on discharge duration. The reduced rate of spike augmentation may have been the consequence of suppressed recurrent excitation between bulbospinal Aug-I neurons (21, 28).

After higher doses of fentanyl, it is likely that a source of tonic excitatory drive to bulbar respiratory neurons was suppressed. It can be assumed that such doses of fentanyl depressed brain stem chemoreceptor neurons that provide generalized tonic excitation to bulbar respiratory neurons (50, 51), since \( \mu \)- and \( \delta \)-opioid receptor agonists blunt respiratory responsiveness to \( \text{CO}_2 \) (32, 56, 63). An additional potential site of opioid-mediated depression is the brain stem reticular activating system (51). Opioids also depress peripheral chemoreceptor neurons (53), but such an action was probably not a major contributor in the present experiments because \( \text{CO}_2 \) was maintained within the normal range for the cat (27) and animals were ventilated with \( \text{O}_2 \)-enriched air.
Respiratory network disturbances by fentanyl are similar in anesthetized and unanesthetized decerebrate preparations. The present results indicate that general anesthesia is permissive rather than obligatory for disturbances of rhythm and excitability caused by systemically administered fentanyl. The disturbances were qualitatively similar in pentobarbital-anesthetized and unanesthetized decerebrate cats. Differences were quantitative, in that pentobarbital-anesthetized cats exhibited a more prolonged inspiratory phase and were rendered apneic by lower doses.

The finding that the disturbances are similar in anesthetized and unanesthetized preparations is important in two respects.

First, it was reported in a review article (53) that opiates stimulate respiration in the cat as well as in some other species and depress respiration only in the presence of general anesthetics or other central nervous system depressants. Under these conditions, breathing has been reported to slow without affecting tidal volume. However, the relevant reference cited (11) refers to effects of much less selective μ-opioid receptor agonists than fentanyl on breathing. Furthermore, the behavioral stimulant property of such opiates in the awake, freely moving animal is a complicating factor. Findings of the present investigation show that anesthesia is not necessary for selective μ-opioid receptor agonists to depress the respiratory network in the cat, in agreement with a recent report (26). Furthermore, both rate and intensity of respiratory activity is depressed with increasing dose in the cat, in common with other species.

Second, reports that opioids increase resistance of the chest wall to expansion and induce upper airway obstruction refer to their occurrence during and after general anesthesia (1, 3, 7, 8, 13, 43, 47, 54). The present investigation indicates that anesthesia is not obligatory for the occurrence of these disturbances. The finding is consistent with the observation (29) that tonic firing of nerve filaments innervating the expiratory muscles of the chest wall, a condition that would promote decreased compliance, occurs in unanesthetized decerebrate rabbits.

**Fig. 12.** Summary of how opioids with selectivity for μ-opioid receptors can reduce tidal volume chest wall compliance and upper airway resistance. Flow diagram depicts how intravenous fentanyl can evoke disturbances of ventilation based on its effects on membrane properties of 6 types of medullary respiratory neurons. Bulbar and peripheral chemoreceptor neurons and reticular activating system (RAS) neurons provide excitatory drive to all of the different types of respiratory neurons. Opioid-evoked presynaptic and postsynaptic depression of excitability in Con-I propriobulbar (Con-I PB) and Aug-I bulbospinal (Aug-I BS) neurons depresses excitability of phrenic and inspiratory intercostal motoneurons leading to reduced tidal volume and rate of inflation. Opioids evoke tonic discharges in Aug-E bulbospinal (Aug-E BS) neurons, leading to reduction of chest wall compliance by presynaptically blocking inhibition and termination of discharges. The disinhibition of Aug-E BS neurons and resultant reduction of compliance also reduces tidal volume. Opioids presynaptically depress excitability of Aug-I vagal neurons to reduce vocal fold adduction and evoke tonic firing of vagal postinspiratory (Post-I Vagal) neurons by presynaptically suppressing inhibitory synaptic drive potentials. These complementary actions promote tonic vocal fold closure and consequently increased airway resistance. Rostral Aug-E neurons involved in constriction of pharyngeal muscles are depressed presynaptically by systemic fentanyl, further increasing upper airway resistance.
Mechanisms that contribute to depression of tidal volume. Measurements made in the present study show that $\mu$-opioid receptor agonists depress excitability of bulbospinal Aug-I neurons and propriobulbar Con-I neurons. The former type of neuron would be expected to excite spinal inspiratory motoneurons, whereas the latter may synaptically activate the former (10, 18, 20), with the end result being reduction of diaphragmatic contraction and depression of tidal volume (Fig. 12). In support of the assumption that reduced phrenic nerve activity is linked to depression of the Aug-I and Con-I neurons, the dose-dependent changes in rhythm and discharge intensity caused by fentanyl in the two types of neurons paralleled those of the phrenic nerve. As evidence that discharges in both types of bulbar inspiratory neurons were depressed by fentanyl postsynaptically, membrane potential was hyperpolarized and neuron input resistance decreased. Further evidence for a $\mu$-opioid receptor-dependent postsynaptic site of action was the depression of discharges in Aug-I bulbospinal neurons by DAMGO applied iontophoretically. The effect of DAMGO and the interpretation thereof are in agreement with studies from other laboratories, which demonstrated that DAMGO iontophoresis depressed glutamate-evoked firing as well as spontaneous discharges in bulbar inspiratory neurons recorded extracellularly (15, 44, 55). Although projection pathways in the earlier studies were not determined, some of the neurons could have been bulbospinal.

Another neuronal mechanism with the potential to reduce tidal volume (Fig. 12) is tonic firing of bulbospinal Aug-E neurons, resulting in resistance of the chest wall to expansion during the inspiratory phase. 

Mechanisms involved in expiratory neuron firing and their impact on chest wall compliance. The effects of fentanyl on membrane properties of Aug-E neurons observed in the present study provide new information that helps to explain how opioids evoke the different dose-related effects on expiratory neuron discharges. Fentanyl reduced the magnitude of inspiratory phase hyperpolarizing SDPs so that discharge duration was lengthened until the neurons fired continuously. The responses were unaccompanied by change of input resistance, which points to a presynaptic site of action, i.e., to disinhibition. The most likely mechanism involves opioid depression of propriobulbar inspiratory neurons that are responsible for early inspiratory and through-inspiratory inhibition of Aug-E bulbospinal neurons (5). Larger doses, in the range of 20–40 $\mu$g/kg, also depressed excitatory SDPs until membrane potential reached a steady level that, although less than peak control frequency, still supported tonic action potential discharges of an intensity that might account for resistance of the chest wall to expansion and rigidity of expiratory abdominal muscles caused by opioids (Fig. 12). The depression of depolarizing SDPs and lower action potential frequency during tonic firing seems to be linked to depression of chemoreceptor neurons, because hypercapnea reversed these opioid-mediated effects.

The abolition of Aug-E neuron discharges and hyperpolarization of membrane potential after 40–60 $\mu$g/kg iv doses of fentanyl occurred without significant change of neuron input resistance. This combination of effects can be explained by at least two general mechanisms.

The first possibility is that the effects were the result of increased hyperpolarizing and decreased depolarizing membrane conductances. DAMGO and other $\mu$-opioid receptor agonists are known to depress excitability of nonrespiratory central nervous system neurons postsynaptically by increasing permeability to potassium ions and decreasing calcium, sodium, and non-specific cationic conductances (2, 25, 61). Thus there may have been no significant net change in somadendritic conductance in Aug-E neurons. Such an explanation, however, raises the question of why a similar combination of membrane permeability changes is not evident in bulbospinal Aug-I or non-antidromically activated Con-I neurons, where hyperpolarization is accompanied by a net increase in membrane input conductance.

The second possibility is that fentanyl produced membrane permeability changes in regions of the dendritic tree that are too distant to be detected by a microelectrode in the soma and that are beyond the reach of somatically applied current pulses. Membrane hyperpolarization due to cationic current flow from soma to dendrites might become evident only after more proximal shunting conductances have been turned off by opioid-mediated presynaptic inhibition. Remote dendritic inhibition is a plausible mechanism because of the anatomic and electrical properties of medullary respiratory neurons; they have widely distributed dendritic trees (9, 36) and receive significant synaptic input at remote as well as proximal regions (36). Moreover, opioid-mediated remote dendritic inhibition has been demonstrated in vitro in locus ceruleus neurons (60).

Opioid effects on vagal postinspiratory neurons, Aug-I motoneurons, and rostral Aug-E neurons account for increased upper airway resistance. Fentanyl prolonged discharges of vagal postinspiratory motoneurons independent of vagus nerve sensory input (62). The site of action seems to be presynaptic within the central nervous system, because the effect on discharge duration took place in conjunction with suppression of inspiratory phase hyperpolarizing synaptic drive potentials without an apparent change of input resistance. Furthermore, iontophoretic application of DAMGO failed to depress neuron discharges.

The membrane hyperpolarization and abolition of firing without change of input resistance produced by larger doses of fentanyl may have been due to inhibition at remote dendritic synapses, for the reasons cited above.

In vagal Aug-I neurons and rostral Aug-E neurons, discharge intensity and duration seemed to be altered by opioids solely through presynaptic effects because fentanyl reduced depolarizing and hyperpolarizing SDPs and firing without changing input resistance,
and iontophoretic application of DAMGO was without effect on vagal Aug-I discharges. Moreover, largest doses of fentanyl did not hyperpolarize membrane potential of either type of neuron, so that remote dendritic inhibition seems unlikely.

The actions of fentanyl on vagal Aug-I and laryngeal postinspiratory neurons are complementary in their potential to promote vocal fold closure. If the rostral Aug-E neurons recorded in this study are vagal pharyngeal motoneurons as their location and membrane properties suggest, significant depression of their firing by fentanyl would further contribute to increased resistance to airflow.

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REFERENCES


