Opiate slowing of feline respiratory rhythm and effects on putative medullary phase-regulating neurons

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Lalley, Peter M. Opiate slowing of feline respiratory rhythm and effects on putative medullary phase-regulating neurons. Am J Physiol Regul Integr Comp Physiol 290: R1387–R1396, 2006. First published November 10, 2005; doi:10.1152/ajpregu.00530.2005.—Opiates have effects on respiratory neurons that depress tidal volume and air exchange, reduce chest wall compliance, and slow rhythm. The most dose-sensitive opioid effect is slowing of the respiratory rhythm through mechanisms that have not been thoroughly investigated. An in vivo dose-response analysis was performed on medullary respiratory neurons of adult cats to investigate two untested hypotheses related to mechanisms of opioid-mediated rhythm slowing: 1) Opiates suppress intrinsic conductances that limit discharge duration in medullary inspiratory and expiratory neurons, and 2) opiates delay the onset and lengthen the duration of discharges postsynaptically in phase-regulating inspiratory and late-inhibitory neurons. In anesthetized and unanesthetized decerebrate cats, a threshold dose (3 μg/kg) of the μ-opioid receptor agonist fentanyl slowed respiratory rhythm by prolonging discharges of inspiratory and expiratory bulbo-spinal neurons. Additional doses (2–4 μg/kg) of fentanyl also lengthened the interburst silent periods in each type of neuron and delayed the rate of membrane depolarization to firing threshold without altering synaptic drive potential amplitude, input resistance, peak action potential frequency, action potential shape, or afterhyperpolarization. Fentanyl also prolonged discharges of postsynaptic inspiratory and late-inhibitory neurons in doses that slowed the rhythm of inspiratory and expiratory neurons without altering peak membrane depolarization and hyperpolarization, input resistance, or action potential properties. The temporal changes evoked in the tested neurons can explain the slowing of network respiratory rhythm, but the lack of significant, direct opioid-mediated membrane effects suggests that actions emanating from other types of upstream bulbar respiratory neurons account for rhythm slowing.

Opiates are well known for their ability to depress ventilation by slowing breathing frequency and reducing tidal volume, gas exchange, upper airway patency, and chest wall compliance (3, 4, 36, 39). They also blunt respiratory network responsiveness to hypoxia and CO2 acidosis (11, 25, 51, 60).

Opiate slowing of respiratory rhythm, leading to arrest of breathing after the highest doses, has been the topic of many experimental investigations (2, 10, 14, 16, 22, 24, 30, 34, 37, 48, 52, 60). According to most recent studies, rhythm slowing seems to be principally related to depression of inspiratory interneurons in the pre-Bötzinger complex (PBC) (15, 34, 37), a region within the ventrolateral respiratory column (VRC) that seems to be critical for generation of respiratory rhythm (40, 52). However, endogenous opioid peptides are distributed throughout the bulbar respiratory network, and opioid receptors are found on many types of respiratory neurons (19, 32–35, 38, 47, 51). Thus, complementary opioid actions on other types of respiratory neurons that contribute to rhythm slowing are also possible. This study was undertaken to investigate two such actions.

In the present study, two previously untested hypotheses related to opioid mechanisms of rhythm slowing were investigated: 1) opiates depress intrinsic membrane conductances that limit discharge length in VRC inspiratory and expiratory neurons and 2) opiates slow the onset and prolong the duration of discharges postsynaptically in VRC late-inspiratory and postinspiratory neurons, the proposed functions of which are to terminate the inspiratory phase and delay the onset of the expiratory phase (12, 42, 43).

A dose-response analysis of the effects of intravenously administered fentanyl, a phenylpiperidine opiate and μ-opioid receptor agonist, on rhythm slowing was carried out in anesthetized and unanesthetized decerebrate adult cats. To estimate whether direct opiate effects on membrane conductances in VRC inspiratory, late-inspiratory, postinspiratory, and expiratory neurons contribute to slowing of respiratory network rhythm, attention was given to the mechanisms by which fentanyl affected membrane potential, input resistance, and action potential threshold, shape, and afterhyperpolarization.

METHODS

Animal preparation. Data were obtained from 10 pentobarbital sodium-anesthetized adult male cats and 3 unanesthetized adult male cats that were decerebrated at the midcollicular level. Care and use of animals were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health and approved by the University of Wisconsin Medical School Animal Care and Utilization Committee. The procedures were previously described in detail (29). The animals were placed in an anesthesia chamber and initially anesthetized with 5% halothane in 100% O2. During halothane anesthesia, the animals were allowed to breathe 100% O2 as long as arterial blood pressure and heart rate remained within the normal range. At least 4 h elapsed between the termination of halothane administration and the beginning of experimentation. Supplemental doses of pentobarbital sodium (4–8 mg/kg) were administered if symptoms indicating significant lightening of anesthesia occurred: 1) spontaneous increases of arterial blood pressure and heart rate, 2) irregular breathing or discharges of phrenic nerve activity (PNA) that decreased

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in duration and increased in frequency. 3) shivering, and 4) movement and cardiorespiratory changes evoked by surgical procedures. The other three cats were maintained under halothane (3–3.5% in O2) anesthesia and decerebrated at the midsagittal level, with removal of brain tissue rostral to the transection, according to the method of Kirsten and St. John (27). Halothane anesthesia was maintained until all surgical procedures were completed. Decerebrate rigidity without evidence of pain or discomfort was evident within 20 min after anesthesia was discontinued.

Surgical procedures performed on anesthetized and decerebrate cats included placement of catheters in one femoral artery to monitor blood pressure and in both femoral veins to administer drugs and infuse Ringer lactate solution for tissue hydration. A cannula was also inserted in the trachea below the larynx to maintain airway ventilation. Animals were mounted in a stereotaxic head holder and suspended by thoracic and lumbar spinal clamps. Blood pressure, tracheal pressure, rate of breathing, body temperature, end-tidal CO2, and inspired O2 were monitored continuously and recorded on chart paper. Temperature was measured rectally and maintained at 36–38°C by external heating. Animals were paralyzed with gallamine triethiodide (4 mg/kg iv initially and 4–8 mg/h thereafter) and mechanically ventilated with O2-enriched (60% O2) room air. Stroke volume of the ventilator was set at 10 ml/kg body wt, and ventilation rate was adjusted to maintain end-tidal CO2 at 4.6–5.3%, which was within the range recorded during spontaneous breathing after production of surgical anesthesia.

Nerve and brain preparation, recording procedures, and measurements. To monitor respiratory network rhythm, phrenic nerves (C5–C6) were exposed bilaterally through a dorsal cervical approach and prepared for recording from their central ends with bipolar silver hook electrodes. PNA was recorded with an alternating-current preamplifier prepared for recording from their central ends with bipolar silver hook electrodes. A cannula was also inserted in the trachea below the larynx to maintain airway ventilation. Animals were mounted in a stereotaxic head holder and suspended by thoracic and lumbar spinal clamps. Blood pressure, tracheal pressure, rate of breathing, body temperature, end-tidal CO2, and inspired O2 were monitored continuously and recorded on chart paper. Temperature was measured rectally and maintained at 36–38°C by external heating. Animals were paralyzed with gallamine triethiodide (4 mg/kg iv initially and 4–8 mg/h thereafter) and mechanically ventilated with O2-enriched (60% O2) room air. Stroke volume of the ventilator was set at 10 ml/kg body wt, and ventilation rate was adjusted to maintain end-tidal CO2 at 4.6–5.3%, which was within the range recorded during spontaneous breathing after production of surgical anesthesia.

Membrane potential was recorded in discontinuous single-electrode approach and prepared for recording from their central ends with bipolar silver hook electrodes. PNA was recorded with an alternating-current preamplifier (Grass Instruments, Quincy, MA; ×2,000 to ×10,000 amplification, band-pass filter 100–3,000 Hz).

Intracellular recordings were obtained from respiratory neurons of the ventrolateral medulla. The head was ventroflexed, the dorsal medulla was exposed by occipital craniotomy, the dura and arachnoid membranes were reflected, and a patch of pia membrane was removed to allow insertion of a glass microelectrode. To provide recording stability, bilateral pneumothorax was performed, and pressure feet were placed gently on the surface of the medulla over and near the site of microelectrode insertion. After pneumothorax, 1–2 cm H2O pressure was applied to the expiratory outflow to prevent atelectasis.

Neurons were impaled with sharp microelectrodes filled with 2 M potassium methylsulfate [direct-current (DC) resistance 60–80 MΩ]. Membrane potential was recorded in discontinuous single-electrode current-clamp mode (SEC-05XL amplifier, npi, Tamm, Germany; bandwidth DC 10 kHz, switching frequency 25 KHz). To test the effects of fentanyl on intrinsic membrane conductances, neuron input resistance and action potential threshold, shape, and afterhyperpolarization were measured. For measurement of input resistance, 60-ms negative-going constant-current pulses were injected through the microelectrode, and the resulting hyperpolarizing voltage drop across the cell membrane was measured.

PNA and neuron membrane potential were displayed on an oscilloscope (Tektronix Instruments, Beaverton, OR) and registered on magnetic tape (Vetron Technology, Rebersburg, PA; DC −5 kHz), as well as on a computer-controlled data acquisition system (PowerLab, AD Instruments, Castle Hill, NSW, Australia; DC −10 kHz) and a paper chart recorder (Gould, Cleveland, OH; DC −10 kHz).

Identification of bulbospinal and vagal respiratory neurons. For identification of bulbospinal inspiratory and expiratory neurons, two concentric coaxial stimulating electrodes (model SNEX-100, AM Systems, Everett, WA) were positioned bilaterally in the cervical reticulospinal tracts at the C1 level. Stimulation with single bilateral shocks (5–15 V, 0.1-ms pulse duration) antidromically activated bulbospinal neurons, as verified by collision with spontaneous action potentials. Axons of vagal respiratory motoneurons were antidromically activated by single shocks (1.5–3 V, 0.1 ms) applied to the central end of the ipsilateral cervical vagus nerve.

Opioid agonist and antagonist administration. Fentanyl citrate (Sigma-Aldrich Chemical, St. Louis, MO) was dissolved in Ringer solution (40 or 80 μg/ml) and administered intravenously in increments of 1 μg/kg at 1-min intervals until a cumulative dose of 10 μg/kg was reached; then additional increments of 2–5 μg/kg were given to determine the size of the dose required to produce additional effects, other than on rhythm, such as depression of discharge intensity (see Fig. 3E).

The μ-opioid receptor antagonist naloxonazine hydrochloride and the δ-opioid receptor antagonist naltrindole hydrochloride (Sigma-Aldrich Chemical) were each dissolved in Ringer solution (0.2 mg/kg) and injected intravenously in a dose of 100 μg/kg.

Euthanasia. Experiments were terminated by intravenous injection of pentobarbital sodium in sufficient quantity (>100 mg/kg) to produce permanent cardiac arrest.

Statistics. Control and test values were evaluated for significance of difference by a paired Student’s t-test using SigmaPlot software (version 4.11, Jandel Scientific). Differences were accepted as significant if P < 0.05. SigmaPlot was also used to derive means ± SE.

RESULTS

The effects of fentanyl on respiratory rhythm, membrane potential, and discharge properties were measured, along with PNA, in 27 neurons of the medulla that were located within the VRC: 6 bulbospinal augmenting inspiratory neurons, 3 nonvagal, nonbulbospinal inspiratory neurons, 9 bulbospinal augmenting expiratory neurons, 3 vagal postinspiratory motoneurons, 3 postinspiratory neurons that were synchronically, but not antidromically, activated by vagus nerve stimulation, and 3 late-discharging inspiratory neurons.

The inspiratory neurons were located 1–2 mm caudal to the obex, 2.5–3.5 mm lateral to the midline, and 2.0–3.0 mm below the dorsal surface of the medulla (Fig. 1). The other neurons were located 1–3 mm rostral, 3.4–3.8 mm lateral, and 3.4–3.8 mm below the dorsal surface. The vagal postinspiratory motoneurons were within the nucleus ambiguus, because they were recorded among antidromically activated inspiratory and expiratory vagal motoneurons that exhibited an augmenting membrane potential and discharge pattern (8). The nonvagal inspiratory neurons and the postinspiratory and late-discharging inspiratory neurons were medial to this region and, therefore, are assumed to be separate from the more laterally located PBC respiratory neurons (50).

All of the neurons exhibited dose-related changes of respiratory rhythm or discharge intensity in <1 min after each effective dose of fentanyl, with no change of heart rate or blood pressure.

Dose-related effects of fentanyl on bulbospinal inspiratory neurons. To test the hypothesis that opioids slow breathing by prolonging discharges of VRC inspiratory and expiratory neurons through depression of intrinsic membrane conductances, effects of fentanyl on temporal properties, neuron input resistance, and action potential properties were measured.

Bulbospinal inspiratory neurons under control conditions exhibited augmenting patterns of membrane depolarization and robust bursts of action potentials that began shortly after the onset of phrenic nerve discharges and continued until PNA entered its declining postinspiratory phase. Thereafter, membrane hyperpolarization occurred and firing ceased during the postsynaptic respiratory rhythm and discharge phases (Fig. 2A, left). Under
control conditions, burst frequency was $20 \pm 0.7\, \text{min}^{-1}$, burst duration was $1.1 \pm 0.04\, \text{s}$, and peak action potential frequency was $42 \pm 2\, \text{s}^{-1}$.

Fentanyl evoked progressive, dose-dependent rhythm slowing. The effects of a greater-than-threshold dose of fentanyl on a bulbospinal inspiratory neuron are shown in Fig. 2A. Effects of three cumulative doses (3, 6, and 10 $\mu$g/kg) of fentanyl on burst frequency, peak action potential frequency, and discharge duration in six inspiratory neurons are summarized in Fig. 2C.

The most sensitive dose-related changes evoked by fentanyl occurred after a cumulative dose of 3 $\mu$g/kg in all the neurons. Discharge duration lengthened to $1.6 \pm 0.07\, \text{s}$ (145% increase, $P < 0.001$), in parallel with prolongation of the phrenic nerve inspiratory phase discharge. This effect reduced burst frequency to $15 \pm 0.6\, \text{min}^{-1}$ (27% decrease, $P < 0.05$). Doses of 3–5 $\mu$g/kg also slowed the rates of membrane depolarization and action potential frequency augmentation (Fig. 2A). Peak action potential frequency at the end of the inspiratory phase decreased, but not significantly, to $38 \pm 1.5\, \text{s}^{-1}$ ($P > 0.05$).

After a total of 6 $\mu$g/kg fentanyl, discharge duration was lengthened to $1.8 \pm 0.1\, \text{s}$ (145% increase, $P < 0.001$), and the rates of membrane depolarization and spike frequency augmentation were further slowed, whereas peak action potential frequency remained unaltered. Another change that contributed to rhythm slowing after this larger cumulative dose of fentanyl was prolongation of the silent period. Prolongation of the inspiratory discharge and the expiratory silent period together

Fig. 1. Locations of tested neurons in the ventrolateral respiratory column (VRC) of the medulla oblongata. Ovoid in upper trace encloses AMB dorsally, where respiratory neurons were recorded intracellularly. Ventrally, it encloses a region where negative-going extracellular field potentials were recorded during mapping. Areas enclosed in boxes define locations of inspiratory, late-discharging inspiratory, postinspiratory (post-I), and expiratory neurons. Amb, nucleus ambiguus; C, caudal cuneate nucleus; CST, corticospinal tract; CUR, cuneate nucleus, rostral division; CX, cuneate nucleus, external division; G, gracile nucleus; IO, inferior olive; LRN, lateral reticular nucleus; P, pyramidal tract; PBC, pre-Bötzinger complex; TS, solitary tract; 5SP, spinal trigeminal nucleus, parvocellular division; 5ST, spinal trigeminal tract.

Fig. 2. Dose-related effects of fentanyl responsible for slowing of rhythm in bulbospinal inspiratory neurons of the ventrolateral respiratory column (VRC). A: slowing of respiratory rhythm in a bulbospinal inspiratory neuron by $\mu$-opioid receptor agonist fentanyl. Top traces, membrane potential (MP); dashed lines, action potential threshold (top) and maximum membrane hyperpolarization (bottom). Bottom traces, electroneurograms of phrenic nerve activity (PNA). B: expanded time records of action potentials. Dashed lines, action potential threshold (top) and maximum afterhyperpolarization (bottom). C: dose-related effects of fentanyl on discharge duration, peak action potential frequency (APs/sec), and discharge frequency (bursts/min) in 6 bulbospinal inspiratory neurons. Lines connect 3, 6, and 10 $\mu$g/kg dose-related data points; other data points (e.g., 1, 2, 4, and 9 $\mu$g/kg) are omitted to simplify presentation. Threshold dose in all experiments was 3 $\mu$g/kg. Abscissa, cumulative doses of intravenously administered fentanyl. Absolute control values are shown at top left.
decreased burst frequency to $11 \pm 1.4$ min$^{-1}$ (46% decrease, $P < 0.001$).

A cumulative 10 $\mu$g/kg dose of fentanyl slowed cycle frequency to $9 \pm 1.2$ min$^{-1}$ (54% decrease, $P < 0.001$) as a result of further prolongation of inspiratory discharges [to $2.5 \pm 0.32$ s (227% increase), $P < 0.001$] and expiratory silent periods. Prolongation of the inspiratory phase discharge duration and the expiratory silent period occurred with no effect on neuron input resistance, firing threshold, or action potential shape, duration, or afterhyperpolarization (Fig. 2B).

After $\geq 10$ $\mu$g/kg doses of fentanyl, effects indicative of postsynaptic depression appeared, as previously described (29). In the six neurons, fentanyl produced hyperpolarization of membrane potential and depression of depolarizing synaptic drive potentials. Peak action potential frequency declined significantly after 10 $\mu$g/kg [to $22 \pm 2.8$ s$^{-1}$ (48% decrease), $P < 0.001$], and neuron input resistance was reduced.

Dose-dependent changes in nonvagal, nonbulbospinal inspiratory neurons. In the VRC, rostral to the bulbospinal inspiratory neurons, inspiratory neurons exhibited plateau-like depolarizing synaptic drive potentials and action potential frequencies that were maximal with the onset of PNA and maintained throughout the inspiratory phase. They were not activated antidromically by spinal cord or vagus nerve stimulation. Dose-dependent fentanyl effects on one of the neurons is illustrated in Fig. 3.

The 3 $\mu$g/kg threshold dose of fentanyl slowed rhythm by lengthening discharge duration (Fig. 3B). Further lengthening of discharge and prolongation of the silent period occurred after larger (6–15 $\mu$g/kg) doses (Fig. 3, C and D), but synaptic drive potential amplitude and action potential shape, threshold, and afterhyperpolarization were not affected.

After cumulative 18–20 $\mu$g/kg doses of fentanyl, slowing of rhythm was accompanied by reduced action potential frequency, increased action potential afterhyperpolarization, and reduced synaptic noise during the postinspiratory and expiratory phases (Fig. 3E).

Fentanyl effects on bulbospinal expiratory neurons. The expiratory neurons of the caudal VRC that were recorded in anesthetized (6 cells) and decerebrate unanesthetized (3 cells) cats exhibited periodic membrane hyperpolarization and absence of firing in parallel with the phrenic nerve discharge during the inspiratory phase. They depolarized when PNA declined during the postinspiratory phase and discharged action potentials during the late expiratory phase in conjunction with the phrenic nerve silent period, as previously described (1).

The dose-related slowing of rhythm by fentanyl recorded in two neurons is illustrated in Figs. 4 and 6, and results from six

![Fig. 3. Dose-related slowing of respiratory rhythm by fentanyl in a nonvagal, nonbulbospinal inspiratory neuron. Top traces, membrane potential and discharge properties. Bottom traces, electroneurograms of PNA. Time scale in A applies to A–E. Doses of fentanyl are cumulative.](#)
neurons are summarized in Fig. 5. Discharge duration before administration of fentanyl was 1.95 ± 0.19 s, burst frequency was 16.1 ± 1.7 min⁻¹, and peak action potential frequency was 56 ± 10.1 s⁻¹.

The threshold dose of fentanyl in all of the neurons, from anesthetized or decerebrate cats, was 3 μg/kg. It increased discharge duration to 2.51 ± 0.25 s (29% increase, P < 0.05), prolonged the silent period in conjunction with longer phrenic nerve inspiratory discharges, and reduced neuron burst frequency to 11.2 ± 1.5 min⁻¹ (30.4% decrease, P < 0.001). Although peak action potential frequency was, on average, increased, the change was not statistically significant.

Increasing the dose of fentanyl further prolonged the discharge duration and the silent period. After a cumulative 6 μg/kg dose, discharge duration lengthened to 4.19 ± 0.85 s (115% increase, P < 0.01) and burst frequency slowed to 8.6 ± 1.7 min⁻¹ (45% decrease, P < 0.001). An additional opioid effect after the total dose reached 9–12 μg/kg was a more gradual depolarization of membrane potential during the postinspiratory phase of PNA (Fig. 4, C and D) (29). Identical dose-related opioid effects were observed in expiratory neurons of unanesthetized decerebrate cats.

Rhythm slowed over the range of 3–12 μg/kg fentanyl without changing synaptic drive potential amplitude or action potential threshold, spike properties, and afterhyperpolarization (Fig. 6D). Input resistance also remained unchanged. Effects on inward rectification, another opiate postsynaptic outcome (58), were also not evident; steady membrane hyperpolarization beyond the reversal level for the inhibitory synaptic drive potential failed to alter neuron input resistance (Fig. 6C).

Doses of fentanyl >12 μg/kg had other effects, including depression of excitatory and inhibitory synaptic drive potential amplitude and marked reduction of action potential frequency (29).

Effects on postinspiratory and late-inspiratory neurons. Measurements were made on late-discharging inspiratory and postinspiratory neurons to test the second hypothesis: that an additional mechanism through which opiates slow breathing is delay of the onset and prolongation of the duration of discharges in VRC late-discharging inspiratory and postinspiratory neurons postsynaptically. There is evidence that the neurons modulate the rate of respiration by terminating inspiratory neuron discharges and that postinspiratory neurons can prolong the expiratory phase silent period and delay the onset of expiratory neuron discharges (12, 18, 21, 43). Thus slowing and prolongation of their discharge properties would imply that they contribute to opioid rhythm slowing, and alteration of action potential properties and input resistance would suggest postsynaptic effects.
It was possible to maintain stable recordings from only a few neurons of each type long enough to test several doses of fentanyl on each cell. Fentanyl was tested on two types of postinspiratory neurons, neither of which responded to spinal cord stimulation. One type, the vagal postinspiratory motoneuron (n/H110053 cells), was activated synaptically, as well as antidromically, by vagus nerve stimulation. The neurons, in the absence of stimulation, hyperpolarized during the phrenic nerve inspiratory discharge and then depolarized to firing threshold on termination of inspiratory PNA and transition to the postinspiratory discharge phase. Membrane potential then dropped below firing threshold during the expiratory phase and depolarized to threshold again just before the next cycle of inspiratory phrenic nerve activity (Fig. 7B1). Single shocks (1.5–3.0 V, 0.1 ms) applied to the ipsilateral vagus nerve evoked constant-latency antidromic action potentials that were followed by excitatory postsynaptic potentials with onset latencies of 5–7 ms and bursts of three to four action potentials (Fig. 7A). Several shocks applied at the end of inspiratory PNA at a rate of 3 Hz produced robust, prolonged postinspiratory neuron discharges. In association with the neuron effects, prolongation of the phrenic nerve postinspiratory discharge and lengthening of the silent period resulted in slowing of the phrenic nerve rhythm (Fig. 7B2).

Fentanyl administration prolonged membrane potential depolarization and discharge and increased discharge intensity in all three cells (Fig. 8, A and B). The threshold dose was 3 μg/kg. A cumulative 6 μg/kg dose prolonged depolarization from 3.6 s (control average) to 6.8 s, increased the postinspiratory discharge duration from 1.2 to 4.4 s, and lengthened the preinspiratory discharge time from 110 to 223 ms. Action potentials and discharge (Fig. 7A and B) were illustrated with 3-Hz single shocks applied to the ipsilateral vagus nerve. Anionic direct current (1 nA dc) was applied in Fig. 7A to illustrate antidromic action potentials followed by synaptic responses. In Fig. 7B, responses were recorded with and without vagus nerve stimulation at a rate of 3 Hz. Dashed lines, spike threshold (top) and afterhyperpolarization (bottom).

Fig. 6. Input resistance and action potential properties are unchanged in bulbospinal expiratory neurons during rhythm slowing by fentanyl. A–C: brief, regularly spaced downward deflections of membrane potential for measurement of neuron input resistance were produced by 60-ms hyperpolarizing constant-current pulses (top traces). In C, membrane potential was hyperpolarized beyond reversal level for inhibitory synaptic drive potential by applied anionic direct current (1 nA dc). Time scale in A applies to A–C. D: action potentials recorded at a faster time scale. Discharges were recorded during peak late-expiratory phase under control conditions (top) and after 6 μg/kg fentanyl (bottom) in the absence of direct-current and hyperpolarizing current pulses. Dashed lines, spike threshold (top) and afterhyperpolarization (bottom).

Fig. 7. Vagus nerve stimulation produces prolongation of discharge in postinspiratory vagal motoneurons, accompanied by respiratory network slowing. A: superimposed traces on an expanded time scale to illustrate antidromic followed by synaptic responses of a vagal postinspiratory neuron (MP; top traces) to 3-Hz single shocks applied to ipsilateral vagus nerve. B: responses recorded on a compressed time scale without (1) and with (2) vagus nerve stimulation. Vagus nerve stimulation lengthens silent period of PNA in conjunction with robust, sustained postinspiratory neuron firing. Silent period and neuron discharge outlast vagus nerve stimulation and terminate simultaneously.
potential frequency was increased from 5 to 19 s\(^{-1}\) during the postinspiratory phase and from 6 to 10 s\(^{-1}\) during the preinspiratory phase. In conjunction with these effects, phrenic nerve inspiratory discharges were prolonged from 1.7 s (control average) to 3.2 s, and expiratory silent periods were lengthened from 2.1 to 5.7 s.

The other type of postinspiratory neuron was nonvagal and nonbulbospinal but was synthetically activated by vagus nerve stimulation. These neurons (\(n = 3\) cells) were located medial to vagal respiratory motoneurons and, therefore, were not part of the PBC. Ipsilateral single shocks evoked excitatory postsynaptic potentials (5.2 ± 0.7 ms onset latency) and action potentials (17.5 ± 5.3 ms) that were accompanied by depression of phrenic nerve discharges (Fig. 9A). Continuous vagus nerve stimulation (not shown) prolonged membrane potential depolarization and discharge duration. Phrenic nerve discharges were shortened, and the silent periods were lengthened sufficiently to slow cycle frequency. The effects of vagus nerve stimulation confirm findings of Hayashi et al. (21) in adult rats in vivo and support their conclusion that the postinspiratory neurons contribute to inspiratory termination.

Responses to fentanyl were similar to those measured in the other type of postinspiratory neuron. The threshold dose (3 µg/kg) prolonged hyperpolarizing and depolarizing synaptic drive potentials and increased discharge intensity and duration (Fig. 9C). These effects increased with dose. After 6 µg/kg, the depolarizing synaptic drive potential duration was lengthened from 1.4 s (control average) to 3.2 s, discharge duration from 0.6 to 3.2 s, action potential frequency from 8.3 to 40 s\(^{-1}\), and duration of the hyperpolarizing synaptic drive potential from 2.1 to 3.3 s. In association with the neuronal effects, phrenic nerve inspiratory discharges were lengthened from 1.5 s (control average) to 3.3 s and the silent period from 2.1 to 10.8 s.

In either type of postinspiratory neuron, input resistance and action potential shape, threshold, and afterhyperpolarization were not altered.

The late-discharging inspiratory neurons (\(n = 3\)), located in the same VRC region where nonvagal postinspiratory neurons were found, exhibited membrane potential and discharge properties similar to those described by Richter (42) and Haji et al. (18). Under control conditions (Fig. 10A), they depolarized at the onset of PNA and discharged briskly late in the inspiratory phase. When the late-discharging inspiratory neurons reached peak action potential frequency, inspiratory PNA ceased and the postinspiratory discharge phase began. They then hyperpolarized until the next cycle of PNA began.

The threshold dose (3 µg/kg) of fentanyl slowed the rate of membrane depolarization, prolonged its duration, lengthened the late inspiratory discharge without altering peak action potential frequency, and increased neuron input resistance (Fig. 10B). These changes increased with dose. After 6 µg/kg, the rate of membrane potential depolarization decreased from 14.2 mV/s (control average) to 5.1 mV/s, discharge duration lengthened from 0.4 to 0.8 s, peak action potential frequency did not change appreciably from the control average of 43 s\(^{-1}\), and input resistance increased from 25 to 33 MΩ. In conjunction with slowing of late-discharging inspiratory neuron rhythm,
OPIOID-MEDIATED RESPIRATORY RHYTHM SLOWING

During all phases of the respiratory cycle, neuron firing and membrane conductances are slowed by fentanyl, and neuron input resistance is increased. Rhythmic membrane depolarization and hyperpolarization in the neuron, along with PNA, are slowed by fentanyl, and neuron input resistance is increased during all phases of the respiratory cycle.

From the in vitro studies, it seems clear that the PBC is a major site where opiate substances slow rhythm (34, 37). However, critical for rhythm generation. Opioid mechanisms of slowing the presence of opioid receptors in areas that are deemed consequences related to opiate overdose, but also because of the presence of opioid receptors in areas that are deemed critical for rhythm generation. Opioid mechanisms of slowing in neonatal in vitro preparations have been reported and applied to theories of respiratory rhythm generation (37, 56). From the in vitro studies, it seems clear that the PBC is a major site where opiate substances slow rhythm (34, 37). However, opiates are known to have actions at other sites in the adult intact respiratory network that contribute to slowing of breathing (20, 23, 24, 55).

The main findings and conclusions derived from this study are as follows: 1) Lengthening of inspiratory and expiratory neuron discharges, effects that slow respiratory rhythm, is not related to opioid postsynaptic actions on any of the VRC types of neurons tested. 2) Other candidate sites of opiate-mediated rhythm slowing, in particular the PBC and the rostral pons, are implicated from the pattern of slowing.

Opioid receptors are present on VRC inspiratory and expiratory neurons but do not appear to contribute to network rhythm slowing. The evident lack of direct effects on inspiratory and expiratory neurons that could contribute to slowing was surprising, because 1) intravenous doses of morphine and fentanyl have postsynaptic effects on the neurons (19, 29), 2) there is evidence that calcium-activated potassium currents shorten discharge duration in VRC inspiratory and expiratory neurons (40, 44), and 3) opioids are known to decrease calcium current through voltage-gated calcium channels in many types of central nervous system neurons (53). Taken together, these findings raise the possibility that opiates lengthen discharge duration in inspiratory and expiratory neurons by depressing intrinsic calcium-activated potassium conductances. Indeed, there was no evidence from measurements of input resistance or action potential threshold, shape, and afterhyperpolarization that the doses of fentanyl that slowed rhythm acted on membrane conductances. Slowing is evidently generated through effects on other neurons that control the onset and termination of inspiratory and expiratory neuron discharges.

Medullary late-discharging inspiratory and postinspiratory neurons do not appear to be directly responsible for opioid slowing of rhythm. Late-discharging inspiratory and postinspiratory neurons, according to the network concept of rhythm control, are responsible for off-switching inspiratory neurons. Postinspiratory neurons, in addition, slow the onset of expiratory neuron discharges and prolong the silent period of PNA (21, 42, 43). A delay in firing of late-discharging and postinspiratory neurons and prolongation of postinspiratory neuron discharges might thus lead to lengthening of the inspiratory and expiratory phases. Fentanyl did indeed delay and prolong late-discharging inspiratory and postinspiratory firing in association with slowing of cycle frequency in inspiratory and expiratory neurons and in PNA. However, input resistance and action potential properties in postinspiratory neurons were unaltered. In late-discharging inspiratory neurons, resistance increased throughout all phases of the respiratory cycle, an opiate effect that might have occurred through reduction of afferent tonic excitatory drive from a number of sources (42, 46). The lengthening of discharge duration can also be explained by an upstream effect: delayed inhibitory synaptic input from postinspiratory neurons (42). Overall, the measurements do not implicate postsynaptic opiate effects on late-discharging inspiratory and postinspiratory neurons in network rhythm slowing.

Properties of rhythm slowing eliminate a few other brain sites of opioid action but implicate the rostral pons and PBC. The lengthening of inspiratory and expiratory phases of the respiratory cycle eliminates a number of potential opioid-mediated mechanisms. For example, opioids enhance Hering-Breuer reflex-mediated slowing of respiration (5, 7, 9, 12, 21,
61), but the effect is due solely to prolongation of the expiratory phase, and, in the present study, the reflex was eliminated by bilateral cervical vagotomy before fentanyl was tested. Respiration is also slowed when N-methyl-D-aspartate (NMDA) receptors are pharmacologically blocked and vagal pulmonary feedback to the central nervous system is prevented (13, 57). Although opioids can suppress NMDA-mediated synaptic transmission (26), NMDA receptor blockade selectively prolongs the inspiratory phase (13, 31). Anesthetics, particularly pentobarbital sodium, slow rhythm (57) and enhance slowing by opioids (48). However, opioid-mediated reductions of cycle frequency occurred within the same range of doses and to similar dose-dependent degrees in anesthetized and unanesthetized decerebrate preparations in the present study.

The pattern of rhythm slowing suggests two potential sites of opiate action in the bulbar respiratory network: thepons, in the regions of the Kolliker-Fuse nucleus (KFN) and medial parabrachial nucleus, and the PBC.

Direct administration of morphine into the KFN and medial parabrachial nucleus regions slows respiratory rhythm by prolonging the inspiratory and expiratory phases (23, 24, 55). There are functional synaptic connections between KFN neurons and ventral respiratory group inspiratory and expiratory neurons (49), and computer modeling of the pontomedullary respiratory network suggests that the timing and patterns of discharge of all the neurons tested for opiate responsiveness in this study can be influenced by excitatory synaptic inputs from pontine neurons (46).

Inspiratory and preinspiratory neurons of the PBC are also potential targets. Opioids slow discharge frequency of PBC inspiratory neurons in the neonatal brain stem preparation, and the neurons project to other areas of the VRC (35, 38). In the cat, a subset, the type 2 preinspiratory neuron, exhibits a biphasic pattern of discharge that consists of a brief burst at the onset of PNA and another at the termination of the phrenic nerve inspiratory discharge (50) (Fig. 7). It was suggested that they play a role in the transition between expiration and inspiration (6, 28, 51). Lengthening of the interval between the biphasic discharges of type 2 neurons is consistent with lengthening of inspiration and expiration by opioids. Opiate effects on feline preinspiratory neurons have not, however, been reported, and, in the more rostral (parafacial) region of the neonatal rodent preparation, preinspiratory neurons that discharge before and immediately after inspiratory motor nerve discharges do not respond to application of μ-opioid receptor agonists (37, 54).

In summary, this study has eliminated several sites in the medullary VRC that might have contributed to slowing of respiratory rhythm by fentanyl. The pattern of slowing suggests that an important site may be the pons. The pons and the neurons of the PBC could be the primary targets for opiate slowing in the adult respiratory network, although additional sites, such as the Bötzing complex, a more rostral component of the VRC, should also be considered (43).

REFERENCES


