Synaptic Inhibition of Cat Phrenic Motoneurons by Internal Intercostal Nerve Stimulation

MARK C. BELLINGHAM
Division of Neuroscience, The John Curtin School of Medical Research, The Australian National University, Canberra, Australian Capital Territory 0200, Australia

Bellingham, Mark C. Synaptic inhibition of cat phrenic motoneurons by internal intercostal nerve stimulation. J. Neurophysiol. 82: 1224–1232, 1999. Intracellular recordings from 65 phrenic motoneurons (PMNs) in the C3 segment and recordings of C5 phrenic nerve activity were made in 27 pentobarbitone-anesthetized, paralyzed, and artificially ventilated adult cats. Inhibition of phrenic nerve activity and PMN membrane potential hyperpolarization (48/55 PMNs tested) was seen after stimulation of the internal intercostal nerve (IIN) at a mean latency to onset of 10.3 ± 2.7 ms. Reversal of IIN-evoked hyperpolarization (n = 14) by injection of negative current or diffusion of chloride ions occurred in six cases, and the hyperpolarization was reduced in seven others. Stimulation of the IIN thus activates chloride-dependent inhibitory synaptic inputs to most PMNs. The inhibitory phrenic nerve response to IIN stimulation was reduced by ipsilateral transection of the lateral white matter at the C3 level and was converted to an excitatory response by complete ipsilateral cord hemisection at the same level. After complete ipsilateral hemisection of the spinal cord at C3 level, stimulation of the IIN evoked both excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) in PMNs (n = 10). It was concluded that IIN stimulation can evoke both excitatory and inhibitory responses in PMNs using purely spinal circuitry, but that excitatory responses are normally suppressed by a descending pathway in intact animals. Fifteen PMNs were tested for possible presynaptic convergence of inputs in these reflex pathways, using test and conditioning stimuli. Significant enhancement (>20%) of IPSPs were seen in seven of eight IIN-evoked responses using pericruciate sensorimotor cortex (SMC) conditioning stimuli, but only one of five IIN-evoked responses were enhanced by superior laryngeal nerve (SLN) conditioning stimuli. The IIN-evoked IPSP was enhanced in one of two motoneurons by stimulation of the contralateral phrenic nerve. It was concluded that presynaptic interneurons were shared by the IIN and SMC pathways, but uncommonly by other pathways. These results indicate that PMNs receive inhibitory synaptic inputs from ascending thoracocervical pathways and from spinal interneurons. These inhibitory reflex pathways activated by afferent inputs from the chest wall may play a significant role in the control of PMN discharge, in parallel with disinhibition following reduced activity in bulbospinal neurons projecting to PMNs.

INTRODUCTION

The inspiratory discharge of the phrenic nerve can be suppressed by stimulation of a number of peripheral or central sites. This response has been best characterized for electrical stimulation of the pericruciate sensorimotor cortex (SMC) (Lipski et al. 1986), the superior laryngeal nerve (SLN) (Bellingham et al. 1989; Biscoe and Sampson 1970; Jodkowski and Berger 1988), and the intercostal nerves (Decima and Euler 1969; Remmers 1973). The suppression of phrenic nerve discharge has been shown to be at least partially due to chloride-dependent inhibitory synaptic inputs to phrenic motoneurons (PMNs) for the cortical and superior laryngeal stimulation sites (Bellingham et al. 1989; Lipski et al. 1986). In contrast, based on the effect of upper cervical cord lesions and the responses of bulbospinal medullary respiratory neurons, it has been proposed that the phrenic nerve response to intercostal nerve stimulation is due to disinhibition of bulbospinal excitatory inputs to PMNs (Bolser et al. 1987; Bolser and Remmers 1989; Remmers 1973; Shannon et al. 1987). To date, this hypothesis has not been confirmed by intracellular recordings of the response of PMNs to stimulation of the intercostal nerves.

Investigation of the intercostal-to-phrenic reflex and the pathway it utilizes may be able to give insights into inhibitory mechanisms controlling PMN discharge. The aim of the experiments reported here was to investigate the effects of internal intercostal nerve (IIN) stimulation on PMNs by intracellular recording of the responses of PMNs. Test and conditioning stimuli, using different stimulation sites known to inhibit PMNs, were then applied, enabling some inferences to be made regarding the presence, degree, and site of convergence in presynaptic pathways. To provide information about the pathways utilized by supraspinally evoked responses, and also the minimum neural substrate necessary for intercostal-to-phrenic reflex responses, selective lesions of descending spinal tracts and high spinalization were done in some animals.

Some of these results have already been published in abstract form (Bellingham 1989).

METHODS

Recordings were made from 27 adult cats of either sex (1.5–2.6 kg). The experimental protocol and animal care during experiments was in accordance with institutional and national guidelines. Animals were anesthetized by injection of pentobarbital sodium (35–40 mg/kg ip); anesthesia was subsequently maintained by injections of the same drug (3–6 mg/h iv). Atropine sulfate (0.04 mg/kg im) and dexamethasone (2 mg im) were given to minimize airway secretions and cerebral edema, respectively. Cannulae were inserted into a femoral vein and artery, for the administration of drugs, and to monitor heart rate and arterial blood pressure (Statham P23). A cannula was inserted into the trachea through a tracheostomy, and expired CO2 levels (Datex Normocap), respiration rate, and tracheal pressure were measured.

The C3 branches of both phrenic nerves, both SLNs and the 6th and/or 7th IIINs were prepared for bipolar recording or stimulation. The SLNs were cut at their entry into the laryngeal muscles, and the...
IINs and their individual muscular filaments were cut as far distally as possible and mounted on bipolar electrodes, without contact to the lateral branch of the IIN or the external intercostal nerve. The animal was held in a stereotaxic frame with ear bars and clamps at T1, T12, and L4 and suspended in the prone position, with little or no support to its thorax or abdomen. A dorsal laminectomy exposed spinal cord segments C6–C7 and the dura was opened. The Atlantooccipital membrane was opened to decrease respiratory and cardiac pulsations of the spinal cord. The pericruciate SMC was exposed by removal of the frontal bones and opening of the dura mater. An Ag–AgCl2 earth electrode was inserted into, or tied onto, nearby neck muscles. All exposed tissues were covered with warm mineral oil.

Bilateral pneumothoraces were done, and the animal was artificially ventilated (50 cycles/min, stroke volume 25–60 ml); lung atelectasis was prevented by an end-tidal pressure of 2–3 cm H2O. Muscular paralysis was induced with gallamine triethiodide (8 mg/kg iv) and maintained with pancuronium bromide (0.03 mg/kg iv). In some animals, intracellular recordings showed artifacts related to respiratory movements. These were abolished by changing from a conventional ventilator to a high-frequency animal ventilator (700–800 cycles/min, 70% duty cycle and 20–60 kPa) (Duffin and Lipski 1986).

Mean arterial blood pressure was maintained above 80 mmHg by administration of 5% dextrose-saline (iv), metaraminol bitrate (0.05 mg im or iv), or norepinephrine (1:250,000 solution) iv, when necessary. End-tidal CO2 was kept between 4 and 6% during recording, at a level that maintained a steady phasic discharge of the phrenic nerve. Arterial blood gas and pH levels were measured (Corning 168 pH/blood gas analyzer) and acidosis corrected by increasing the ventilation volume and/or intravenous administration of 1 mM/ml sodium bicarbonate solution, according to calculated base excess values. Rectal temperature was measured and maintained at 38 ± 0.7°C by a feedback circuit to a heating blanket.

The depth of anesthesia was maintained at levels sufficient to prevent pain during all surgical and recording procedures. Before muscle paralysis, an adequate depth of anesthesia was determined by suitable reflex responses (palpebral and withdrawal reflexes absent, corneal reflex present), and the absence of sudden changes in pulse or respiratory rates, arterial blood pressure, or pupil size, in response to painful stimuli. After paralysis, depth was judged by lack of sudden changes in monitored signals, particularly blood pressure, pulse rate, and phrenic nerve discharge. Paralysis was also periodically allowed to wear off, and reflex responses were checked. At the conclusion of experiments, the animal was killed by intravenous overdose with pentobarbital sodium.

The activity of the phrenic nerves was band-pass filtered (30 Hz to 3 kHz) and amplified (Tektronix 5A18N); this amplified activity could be averaged during inspiration by computer. Phrenic nerve activity was also integrated, full wave rectified (3rd order Paynter filter, time constant 100 ms) and displayed on a pen recorder (Neotrace 400ZF).

Stimulation was by constant voltage stimulators (Digitimer DS2, 3–4 Hz) and amplified (Tektronix 5A18N); this amplified activity could be averaged during inspiration by computer. Phrenic nerve activity was also integrated, full wave rectified (3rd order Paynter filter, time constant 100 ms) and displayed on a pen recorder (Neotrace 400ZF).

The use of two subthreshold stimuli to evoke a motoneuronal response proved to be inconvenient, because the establishment of subthreshold levels of stimulation required many averaging trials. Suprathreshold stimuli were thus usually used, and the presence of significant excitatory convergence on common interneurons was inferred when the postsynaptic potential from combined stimulation was 20% larger than the sum of postsynaptic potentials evoked by separate stimuli. This level was arbitrarily chosen, to allow for fluctuation in the postsynaptic potential amplitude due to minor changes in membrane potential and the effects of PMN phasic membrane potential shifts.

In four experiments, the cervical laminectomy was extended to the C6–C7 junction, and transverse lesions were made in the C2–C3 and the caudal part of C4 segments with fine pointed watch-makers forceps and with a fine point scalpel. These animals received 5% dextrose/saline solution intravenously, at a rate of 25–50 ml/h for at least 2 h before cord lesions, and an intravenous infusion of norepinephrine diluted 1:250,000 in 0.9% NaCl, which started a few minutes before cord lesions, to minimize the drop in blood pressure that occurs with high spinalization. To confirm the position of and extent of lesions made during experiments, 1,000 units of heparin was injected intravenously before the end of the experiment, the tissue of interest excised and placed in a solution of 4% paraformaldehyde in 0.9% NaCl. Tissue blocks were left in this fixative for 2 wk, then cleaned,
nerve inhibition.

The latency from stimulus to onset of PMN responses and peak amplitude of PMN responses were measured. In the case of a biphasic response, these parameters were measured separately for each phase. For the later phase, onset was arbitrarily defined as the point at which membrane potential recrossed the baseline, so that latency to onset may be overestimated for these measurements. Where a train of stimuli were used to evoke a response, latency measurements were made from the final stimulus, because trains of stimuli were only used when responses were weak or absent with single stimuli and train length was kept to the minimum necessary for eliciting a response. Statistical significance was determined by Student’s unpaired t-test and accepted at \( P < 0.05 \).

**RESULTS**

**Phrenic nerve responses to IIN stimulation**

The threshold for phrenic nerve inhibition by stimulation of the IIN ranged from 0.3 to 40 V (mean = 6.5 V for ipsilateral IIN and 11.3 V for contralateral IIN). Threshold levels >10 V (Bolser and Remmers 1989) were only seen in five animals; phrenic nerve response to IIN stimulation was not as marked in these animals.

Inhibition of phrenic nerve discharge was seen after stimulation of the ipsi- or contralateral 6th or 7th IIN. The mean latency to onset of inhibition after stimulation of the 6th IIN was 14.5 ± 1.8 ms (mean ± SD; ipsilateral, \( n = 5 \)) and 19.1 ± 7.7 ms (contralateral, \( n = 3 \)), whereas stimulation of the 7th IIN gave a mean latency to onset of 14.1 ± 3.7 ms (ipsilateral, \( n = 33 \)) and 14.4 ± 2.8 ms (contralateral, \( n = 26 \)). The mean onset latencies were not significantly different for ipsi- versus contralateral stimulation or for the 6th versus 7th IIN (\( P > 0.05 \)). Stimulation of the 7th IIN at levels between 1 and 2 times phrenic nerve response threshold resulted in inhibition, with a mean latency to onset of 13.7 ± 1.7 ms (ipsilateral stimulation, \( n = 22 \)) and 13.7 ± 2.4 ms (contralateral stimulation, \( n = 12 \)). Duration of the inhibitory response ranged from 6 to 47 ms. Higher levels of stimulation increased the duration of phrenic nerve inhibition, with no shortening of latency to onset of the response (Fig. 1). Excitatory phrenic nerve responses preceding the inhibition were seen in three cases at high stimulus levels.

C₅ segment cord dorsum potentials evoked by stimulation of the IIN were negative-going potentials of small amplitude. The mean latency from 7th IIN stimulus to onset of the C₅ cord dorsum potential was 7.2 ± 1.1 ms for ipsilateral stimulation (\( n = 6 \)) and 6.3 ± 0.5 ms for contralateral stimulation (\( n = 4 \)). C₅ cord dorsum potentials were not present at IIN stimulus intensities that were subthreshold for phrenic nerve inhibition. The onset of the C₅ cord dorsum potential preceded the onset of phrenic nerve inhibition by a mean of 7.3 ± 1.5 ms (ipsilateral stimulation) and 8.1 ± 2.9 ms (contralateral stimulation). Examples of phrenic nerve and C₅ cord dorsum potential responses to IIN stimulation in the same animal are shown in Fig. 1, illustrating the relationship between the intensity of IIN stimulation, size of the cord dorsum potential, and phrenic nerve inhibition.

**PMN responses to internal intercostal nerve stimulation**

A total of 65 PMNs were tested for response to stimulation of the IIN. Fifty-five PMNs were tested for response to stimulation of the IIN (\( n = 14 \) for ipsilateral 6th IIN, \( n = 37 \) for ipsilateral 7th IIN, \( n = 4 \) for contralateral 7th IIN) in animals with intact spinal cords. In 48 of these PMNs, stimulation of the IIN evoked only a hyperpolarizing response. An example of a hyperpolarization evoked in a PMN by stimulation of the ipsilateral 7th IIN is shown in Fig. 2, together with records of ipsilateral and contralateral phrenic nerve discharge from the same animal. This figure shows the close parallel between the onset of PMN hyperpolarization and phrenic nerve inhibition.

Attempts were made to reverse the polarity of IIN-evoked hyperpolarizations in 13 PMNs. A full reversal was seen in six PMNs and a decrease in the amplitude of hyperpolarization in seven PMNs. Five of the reversal attempts were made with microelectrodes that did not contain chloride ions, resulting in two reversals and three decreases in the amplitude of hyperpolarization during continuous injection of 10–32 nA of hyperpolarizing current, whereas the remainder were with microelectrodes containing chloride ions. These hyperpolarizing PMN responses to IIN stimulation are thus IPSPs. Figure 3 shows examples of the reversal of IIN-evoked IPSPs. Note that in Fig. 3A, the amplitude of the IPSP is diminished after a long period of impalement with a microelectrode containing chloride ions, and reversal was achieved with a low level of current injection compared with the other reversal shown; this was
typical when microelectrodes containing chloride ions were used for recording. Reversal of IPSPs was sometimes incomplete, as in Fig. 3B, in which the peak of the IPSP is still hyperpolarizing, despite reversal of the rising and decaying phase of the EPSP.

Four PMNs showed a biphasic response to stimulation, consisting of depolarization, followed by hyperpolarization. In three of these PMNs, biphasic responses could be converted to hyperpolarizing responses by decreasing the IIN stimulus strength; an example of this is shown in Fig. 4. One PMN responded to stimulation with depolarization alone; the recording microelectrode contained chloride ions, and thus this response may have been a reversed IPSP.

Mean measurements of the postsynaptic potentials are summarized in Table 1 for the different stimulus sites and types of responses. Comparison of the mean latency to onset and amplitude of IPSPs evoked by stimulation of the 6th or 7th ipsilateral IIN found no significant differences for any of these measurements ($P > 0.05$). IPSPs evoked by stimulation of the contralateral 7th IIN had significantly longer latency to onset than IPSPs evoked by stimulation of the ipsilateral 7th IIN ($P < 0.01$).

To determine whether high IIN stimulus levels evoked additional responses by recruiting higher threshold afferents, the IPSPs recorded in this study were divided into two groups, being IPSPs elicited by stimulation from 1 to 2 times threshold for ipsilateral phrenic nerve response, and those elicited by stimulation >2 times threshold (note that this did not mean that the latter group could not be evoked by lower levels of stimulation). Comparison of the mean measurements of the IPSPs from these two groups found no significant difference ($P > 0.05$), with the exception of IPSP amplitude, which was significantly larger ($P < 0.01$) for IPSPs elicited by IIN stimulation >2 times threshold.

**FIG. 2.** Comparison of time course of inhibition of phrenic nerve activity and of the inhibitory postsynaptic potential (IPSP) evoked in a phrenic motoneuron (PMN) by stimulation of the 6th internal intercostal nerve (IIN; 1.2 V, 0.2 ms, 4 Hz) in the same animal. A and B: averages (40 trials) of contralateral (A) and ipsilateral (B) phrenic nerve activity after stimulation in inspiration. C: average of PMN membrane potential (80 responses) to stimulation of the IIN throughout the respiratory cycle.

**FIG. 3.** Hyperpolarizing responses of PMNs to IIN stimulation are IPSPs; intracellular injection of chloride ions and hyperpolarizing current results in reversal of IIN-evoked responses. A: intracellular injection of chloride ions reduces evoked hyperpolarization and the amount of hyperpolarizing current required to reverse the response to IIN stimulation. Two control responses are shown; the 2nd after 40 min of recording with a microelectrode containing 1 M KCl, showing the reduced response. The other record shows reversal of the response while passing a continuous current of $-1.5$ nA. B: control response to IIN stimulation and its reversal during passage of a continuous current of $-14$ nA by a microelectrode filled with 2 M potassium acetate. Note the incomplete reversal of the response. C: control response to IIN stimulation and its reversal during passage of a continuous current of $-22$ nA by a microelectrode filled with a mixture of 2 M potassium acetate and 1 M potassium chloride. All records are averages of 80 (A and C) or 240 (B) responses to ipsilateral 7th IIN stimulation at 4 Hz throughout the respiratory cycle.
Effects of spinal cord lesions on phrenic nerve activity and C₅ cord dorsum potentials

The effects of cervical spinal cord lesions rostral to the C₅ segment on phrenic nerve responses to IIN stimulation were studied in four animals. Transverse cuts in the ipsilateral dorsal column and dorsolateral funiculus at C₂/C₃ or C₄/C₅ levels had no effect on the phrenic nerve response to IIN stimulation. Subsequent complete sectioning of the ipsilateral lateral white matter at C₃ level, sparing the ventromedial quadrant of the white matter, resulted in a decrease in phrenic nerve inhibition in response to IIN stimulation, but did not completely abolish the response. Complete ipsilateral cord hemisection and contralateral dorsomedial column section at C₃ level altered the inhibitory phrenic nerve response to IIN stimulation to a short burst of excitation, which occurred at a similar latency to that of the previously observed inhibition. C₅ cord dorsum potential responses to ipsilateral IIN stimulation were still present after ipsilateral hemisection at C₃/C₄ levels, although their amplitude was reduced.

Intracellular recordings of PMN responses following spinal lesions or transections

Ten PMNs, recorded from two animals, were tested for responses to stimulation of the ipsilateral 7th IIN, following higher spinal lesions that had abolished the inhibitory phrenic nerve response to the same stimulus. Of these 10, 3 PMNs were recorded after complete ipsilateral hemisection and contralateral section of the dorsomedial white matter at C₂/C₃ level. No inspiratory discharge of the ipsilateral phrenic nerve remained after these lesions, and stimulation of the ipsilateral IIN evoked a brief burst of excitation of the phrenic nerve. These PMNs showed small amplitude (1–2 mV) rhythmic depolarization without firing, at the same frequency as respiratory rhythm before spinal lesion. All three motoneurons responded to stimulation of the ipsilateral IIN with excitatory postsynaptic potentials (EPSPs). The other seven PMNs were recorded after a total spinal transection at the junction of the C₂/C₃ segments; no inspiratory discharge of the ipsilateral phrenic nerve remained, and stimulation of the ipsilateral IIN did not evoke a phrenic nerve response. One of these motoneurons responded to stimulation of the ipsilateral 7th IIN with an EPSP, three with EPSPs followed by IPSPs, two with IPSPs, and one failed to respond. The measurements of these PMN responses are shown in Table 1. The mean latency of EPSPs was 5.2 ± 0.3 ms. Comparison of EPSPs from intact and lesioned animals found no significant difference for any EPSP parameter (P > 0.05). The mean onset latency of responses consisting of IPSPs only in spinalized animals was similar to that of IPSPs in intact animals. Examples of the responses evoked in PMNs by IIN stimulation in animals with spinal lesions are shown in Fig. 5. All recordings in these animals were made with microelectrodes without chloride ions; and membrane potential of the PMNs was positive to -270 mV, discounting the possibility that the depolarizing responses recorded were reversed IPSPs.

<table>
<thead>
<tr>
<th>Stimulation Site</th>
<th>Side</th>
<th>N</th>
<th>Response Type</th>
<th>n</th>
<th>Onset Latency, ms</th>
<th>Amplitude, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>6th IIN</td>
<td>Ipsi</td>
<td>14</td>
<td>IPSP</td>
<td>10</td>
<td>11.0 ± 2.7</td>
<td>1.61 ± 0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IPSP(Bi)</td>
<td>2</td>
<td>22.0 ± 2.6</td>
<td>1.48 ± 0.25</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>EPSP*</td>
<td>3</td>
<td>8.1 ± 2.0</td>
<td>0.74 ± 0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th IIN</td>
<td>Ipsi</td>
<td>37</td>
<td>IPSP</td>
<td>34</td>
<td>10.3 ± 2.7</td>
<td>1.24 ± 0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IPSP(Bi)</td>
<td>2</td>
<td>16.6 ± 0.6</td>
<td>1.53 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EPSP*</td>
<td>3</td>
<td>8.2 ± 4.2</td>
<td>1.36 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>Contra</td>
<td>4</td>
<td>IPSP</td>
<td>4</td>
<td>14.8 ± 1.5</td>
<td>1.35 ± 1.24</td>
</tr>
</tbody>
</table>

Spinal transection at C₃ level

| 7th IIN          | Ipsi | 10  | IPSP          |  2| 12.5 ± 1.6      | 1.03 ± 0.50  |
|                  |      |     | IPSP(Bi)      |  3| 28.1 ± 3.4      | 0.50 ± 0.30  |
|                  |      |     | EPSP*         |  7|  5.2 ± 0.3      | 1.87 ± 0.91  |
|                  |      |     | None          |  1|                  |              |

Values in Onset Latency and Amplitude are means ± SD. N is total number of PMNs, and n is number of motoneurons. PMNs, phrenic motoneurons; IIN, internal intercostal nerve; Ipsi, ipsilateral; Contra, contralateral; IPSP(Bi), IPSP of EPSP/IPSP response; IPSP and EPSP, inhibitory and excitatory postsynaptic potential, respectively. * EPSPs and EPSPs of EPSP/IPSP responses grouped together.
**Conditioning trials of PMN responses**

Fifteen PMNs were tested for convergence of IPSPs evoked by stimulation of different inhibitory pathways onto putative common premotor interneurons using test and conditioning stimulation of the contralateral SMC, ipsilateral SLN, and contralateral phrenic nerve. All recordings were made with microelectrodes containing 2 M potassium methylsulfate to eliminate effects of chloride ion diffusion on IPSP amplitude.

Convergence in pathways from the ipsilateral 7th IIN and contralateral SMC was tested for eight PMNs. Using the contralateral SMC as the conditioning stimulus, significant increases (≥20%) in conditioned IPSP amplitude were seen in seven of eight PMNs tested, when SMC stimuli were delivered 3.5–9.5 ms (mean, 5.9 ± 1.5 ms) before the onset of the test stimulus response. Figure 6A shows the changes in conditioned IPSP amplitude for two of these motoneurons over a range of conditioning-to-test stimulus intervals.

Five PMNs were tested for convergence in pathways from the ipsilateral 7th IIN and SLN, using the SLN as the condi-

![Figure 5](image)

**FIG. 5.** Intracellularly recorded responses of 3 different PMNs to IIN stimulation, in an animal with complete spinal transection at C 2 level, showing the range of inhibitory (A), biphasic (B), or excitatory (C) responses. Each record shows C 5 cord dorsum potential (top) and PMN membrane potential (bottom). Stimulation is 3 × 0.2 ms at 1-ms intervals at 4 Hz and 80 responses are averaged. Stimuli are marked with arrows. Calibration is 5 ms (time), 1 mV (membrane potential), and 10 μV (cord dorsum potential).

![Figure 6](image)

**FIG. 6.** Inhibition of PMNs by stimulation of different sites may utilize common premotor interneurons. Stimulation of 2 different inhibitory pathways can cause supralinear summation of evoked IPSPs in PMNs. Amplitude of the conditioned IPSP is expressed as a proportion of the algebraically summed IPSP from separate responses to test and conditioning stimuli (normalized to 1.0); summation in presynaptic interneurons was held to occur when the conditioned IPSP amplitude was 20% greater than the summed IPSP; this level is indicated by the dashed line at 1.2 in each graph. The conditioning stimulus time is expressed as time (in ms) relative to the onset of the IPSP evoked by the test stimulus. A: ipsilateral 7th IIN as the test stimulus and the contralateral SMC as the conditioning stimulus (2 PMNs). B: ipsilateral 7th IIN as the test stimulus and the ipsilateral SLN as the conditioning stimulus (1 PMN). All recordings were made with microelectrodes containing 2 M potassium methylsulfate and are averages of 80–240 responses to stimulation throughout the respiratory cycle.

**DISCUSSION**

The supression of phrenic nerve discharge resulting from stimulation of the IIN seen in this study is similar in latency and duration to previous observations (Decima and Euler 1969;
This response has been thought to be due to disfacilitation of supraspinal inputs to PMNs (Bolser and Remmers 1989; Remmers 1973). Virtually all PMNs tested in the present experiments in animals with intact spinal cords responded to stimulation of the IIN with hyperpolarization, which paralleled the time course of phrenic nerve inhibition evoked by the same stimulus. The reversal of this response, or its decrease in amplitude, by injection of hyperpolarizing current and/or diffusion of chloride ions in the majority of tested PMNs shows that this response is at least partially due to inhibitory synaptic input. Thus although disfacilitation of excitatory inputs from medullary bulbospinal neurons to PMNs certainly does occur (Bolser and Remmers 1989), IIN stimulation also activates inhibitory synaptic inputs to PMNs.

Failure to completely reverse all IIN-evoked IPSPs may be due to simultaneous disfacilitation of PMNs, because the effect of hyperpolarizing current injection on a disfacilitatory response is to increase the amplitude of the response (Llinás and Terzuolo 1964). However, it may also be due to a distal location of synaptic inputs on the dendritic tree (Burke et al. 1971). Because electron micrographs of the phrenic nucleus in the cat show few axosomatic but abundant axodendritic synapses (Takahashi and Ninomyia 1985), it is likely that the reversal of IPSPs in PMNs using hyperpolarizing current injection alone will be relatively difficult, and, in this study, continuous current injection of more than \(-10\) nA was usually necessary to reverse IPSPs when chloride ions were not present in the microelectrode. The failure of the later portion of the IPSP to reverse, seen in some IIN IPSPs, may be attributed either to the presence of two spatially distinct sites of synaptic input (Burke et al. 1971), or, more likely, to concurrent synaptic inhibition and disfacilitation, as is seen in the response of some medullary respiratory neurons to IIN stimulation (Bolser and Remmers 1989).

The onset latencies seen for IIN postsynaptic potentials in both intact and spinal preparations indicate that PMN inhibition and excitation occurs via polysynaptic pathways. One synaptic relay must occur in thoracic segments, as it has been shown that intercostal afferents do not project more than one segment rostral to their segment of entry (Cervero and Connell 1984). The delay between arrival of IIN afferent impulses and PMN response, shown by the delay between the onset of the IIN-evoked C5 cord dorsal potential and that of PMN IPSPs, suggests that one or more interneuronal link in the IIN reflex pathway is also present within the cervical spinal segment. The recording of polysynaptic potentials evoked by IIN stimulation from PMNs in cats with high spinal transections indicate that purely spinal pathways can elicit both EPSPs and IPSPs in PMNs in response to IIN stimulation. Thus although supraspinal disfacilitation of PMNs undoubtedly occurs, it does so in parallel with synaptic input to the PMNs via spinal pathways (Fig. 7).

Although the data presented here establishes that part of PMN responses to IIN stimulation occurs via spinal cord circuits, ascending pathways activated by afferent impulses from the IIN probably ascend to the medulla via the ipsilateral white matter, as for the external intercostal nerves (Remmers 1973). The location of any descending projection from supraspinal neurons activated by IIN stimulation is certainly ipsilateral, and probably in the ventrolateral/ventral white matter, as shown by the lesioning experiments described here. The conversion of the inhibitory phrenic nerve response to one of excitation, and the higher incidence of EPSPs recorded from PMNs in cats with extensive spinal lesions or transection rostral to the C5 segment, may best be explained by a descending ipsilateral pathway, which would normally inhibit the spinal interneurons that produce these EPSPs. Such a pathway might be tonic and excite local segmental inhibitory interneurons, or might be activated by IIN stimulation. The interruption of this inhibitory pathway, or the use of higher intensity stimulation, could then release these excitatory neurons from inhibition. The neurons giving rise to this descending pathway may be located either in the medulla or the upper cervical segments. The response of PMNs to IIN stimulation in the intact cat may thus be an interaction between excitatory and inhibitory inputs, with inhibition predominant. The extent to which the afferents in any intercostal segment activate this descending inhibitory input to segmental interneurons would then determine the phrenic nerve response, consistent with the more prominent excitatory phrenic nerve responses seen when caudal intercostal nerves are stimulated (Decima and Euler 1969; Remmers 1973).

The IIN stimulus levels used in this study were sometimes higher than those used in other studies (Bolser et al. 1987; Bolser and Remmers 1989; Shannon et al. 1987). It is thought that the few cases in which a high threshold for response to SLN or IIN stimulation was seen were due to deeper levels of anesthesia that tend to suppress interneuronal activity. It is unlikely that higher stimulus levels activated additional or separate pathways by activation of higher threshold afferents, because electrical stimulation of the IIN at levels sufficient to excite group I and II fibers has...
been shown to cause suppression of phrenic nerve activity and medullary respiratory neuron discharge, whereas higher levels of stimulation that recruit group III fibers do not contribute to the inhibitory response elicited by group I and II fibers (Shannon 1986). Selective mechanical stimulation of group Ia, Ib, and II nerve endings has shown that responses are mediated primarily by activation of Ib fibers (Bolser et al. 1987; Shannon et al. 1987).

The IINs are mixed nerves that contain both muscle and cutaneous afferent fibers (Shannon 1986). It is possible that stimulation of afferent fibers of mechanosensitive free nerve endings and paciniform corpuscles, which have conduction velocities in the group I and II range, may also be involved in eliciting inhibitory responses (Bolser et al. 1987), but these are few in number and are unlikely to be entirely responsible for the inhibitory effects. Their recruitment, and recruitment of higher threshold Ib afferents, may be responsible for the enhanced phrenic nerve inhibition seen with higher stimulus levels (Remmers 1973; Shannon 1986) and the increased IPSP amplitudes seen in this study at higher stimulus levels. It is thus thought that, whereas the higher levels of stimulation occasionally necessary in this study could excite group II and III afferents, stimulation of these afferents had no significant effects on the responses observed in phrenic nerve activity and intracellular PMN recordings. This is supported by the failure in this study to observe any substantial differences in measurements of responses evoked at low and high levels of stimulation and by the latency of the responses, which are too short to be due to stimulation of slower conducting group II and III afferents.

These results strongly suggest that these responses to IIN stimulation utilize polysynaptic pathways with interneuronal links at spinal or medullary levels (Fig. 7). Stimulation of the SMC or SLN also cause synaptic inhibition of PMNs through medullary and spinal polysynaptic paths (Bellingham et al. 1989; Lipski et al. 1986). Tests for convergence onto common interneurons in these inhibitory pathways to PMNs reveal that some pathways do share presynaptic interneurons. Convergence was most frequent (64%) when SMC conditioning stimuli were used, but was also seen with SLN or contralateral phrenic nerve conditioning stimuli. Because all of these pathways are polysynaptic, with the number and location of interneurons unknown, it is difficult to know at which location activation of common interneurons occurs, but the timing of convergence allows us to estimate some probable locations. Convergence in the SMC and IIN pathways occurs when the SMC stimulus is given ~4–10 ms before the onset of the IIN IPSP, at approximately the same time as the onset of the cord dorsum potential recorded from the C5 segment following IIN stimulation, and so it is possible that common interneurons for these pathways are located in this segment. Similarly, convergence between IIN and contralateral phrenic nerve pathways is most likely to occur at the segmental level, because the phrenic to phrenic reflex persists in high spinal animals (Gill and Kuno 1963).

It thus appears that inhibitory inputs to PMNs may originate at segmental levels, outside of the pool of medullary respiratory neurons. The location and discharge patterns of such inhibitory neurons is of great interest, given their ubiquitous inhibitory actions on PMNs. One question that immediately arises is that of the function of these inputs. The existence of a segmental interneuronal network integrating various inputs to respiratory motoneurons, including descending respiratory drive from medullary neurons, has been suggested (Aminoff and Sears 1971; Davies et al. 1985). The intercostal motoneuron pools receive inputs from both respiratory (Kirkwood et al. 1988) and nonrespiratory interneurons (Sears 1964b), whereas recordings from respiratory phased interneurons have been made from segments containing PMNs in the cat (Bellingham and Lipski 1990; Douse and Duffin 1993; Grélot et al. 1993). The PMN pool thus may also receive inputs from segmental respiratory or nonrespiratory interneurons that integrate and amplify inputs from various peripheral and central sites, in a manner similar to the intercostal motoneuron pools. In addition, the processing of afferent feedback from the chest wall by an interneuronal segmental spinal network could allow this peripheral feedback signal to influence respiratory motor output on a breath-by-breath basis in the awake animal without alteration of the generation of respiratory rhythm.

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