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# The excitability and rhythm of medullary respiratory neurons in the cat are altered by the serotonin receptor agonist 5-methoxy-*N*,*N*, dimethyltryptamine

Peter M. Lalley *

*Department of Physiology, University of Wisconsin, 121 Service Memorial Institutes, 1300 University Avenue, Madison, WI 53706, USA*

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**Abstract**

5-Methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT) is an indolealkylamine which has agonist activity at 5HT receptors. In the present investigation, 5-MeODMT had two types of effects on medullary respiratory neurons of the cat. Iontophoretic administration or i.v. doses ($43 \pm 8.9$ $\mu$g/kg) of 5-MeODMT hyperpolarized respiratory neurons and severely reduced action potential discharges. Cinanserin, a 5HT-2/1 c receptor antagonist, when injected i.v. reduced the inhibition produced by i.v. injection of 5-MeODMT. Iontophoresis of cinanserin did not antagonize inhibition produced by iontophoresis of 5-MeODMT or 5-HT. The depression of respiratory discharge by i.v. injection of 5-MeODMT is attributed to presynaptic effects (network depression) and post-synaptic activation of 5HT-1A receptors on respiratory neurons. 5-MeODMT ($27 \pm 2.78$ $\mu$g/kg i.v.) also increased discharge frequency of inspiratory and expiratory neurons. Inspiratory neuron discharges were briefer and expiratory neuron discharges occurred earlier in relation to phrenic nerve activity. It is suggested that the effects of the smaller doses are due to binding of 5-MeODMT to 5HT-1A receptors on early inspiratory neurons of the medulla.

*Key words:* 5-Methoxy-*N*,*N*-dimethyltryptamine (5 MeODMT); Serotonin receptor; Respiratory neuron; Tachypneic discharge; Respiratory inhibition

## 1. Introduction

The act of breathing is principally controlled by a network of rhythmically active neurons in the brainstem and spinal cord [15,22,32,36–38]. The rhythm of these neurons is established by synaptic interactions and intrinsic membrane properties [15,29,30,36–38] which are controlled by various neurotransmitters and neuromodulators, and can be altered by various drugs. Serotonin (5HT), for example, is an important neuromodulator of respiration [6,20,28,32,33,35]. Many clinically useful drugs [7], as well as drugs of abuse such as LSD and other hallucinogenic indolealkylamines, have actions at 5HT receptors [1] and can alter breathing [34]. The indolealkylamine, 5-methoxy-*N*,*N*,dimethyltryptamine (5-MeODMT), is a 5HT receptor agonist [10] which depresses breathing [24] and respiratory neural discharges recorded from the phrenic nerve [16]. Responses to 5-MeODMT of the various types of neurons which control respiratory rhythm have not heretofore been described, so the mechanism(s) responsible for respiratory depression are unknown. Furthermore, the actions of 5-MeODMT on other types of neurons, such as facial motoneurons [25,42] and neurons which regulate sympathetic vasomotor [26,27] and urinary bladder reflexes [41], do not adequately explain the depression of respiration. A knowledge of how 5-MeODMT acts at cellular and network levels may be useful in understanding how drugs which act at serotoninergic synapses lead to disturbances of respiration.

In the present investigation responses of various types of medullary respiratory neurons to 5-MeODMT administered by iontophoresis, intravenously and by application to the dorsal surface of the medulla, were recorded. It was found that 5-MeODMT has actions which can either reduce discharges of medullary respiratory neurons and the phrenic nerve, or increase the frequency and alter the timing of discharges, depending on dose and route of administration.

* Corresponding author. Fax: (1) (608) 262-2327.


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## 2. Materials and methods

### 2.1. Surgical preparation

Fifty-four cats of either sex weighing between 2.5 and 5.5 kg were anesthetized with pentobarbital sodium (40 mg/kg i.p. initial dose, followed by 4–8 mg/h i.v.) and given atropine methyl bromide (0.2 mg/kg i.v.) to reduce salivation and dexamethasone (0.2 mg/kg i.m.) to prevent brain edema. The trachea was cannulated for artificial ventilation, the femoral veins were catheterized for injection of fluids and drugs and a femoral artery was cannulated for measurement of blood pressure. After fixation of the head and vertebral ($T_1$ and $L_5$) processes in a stereotoxic frame, the medulla was exposed by occipital craniotomy, opening of the dura and reflexion of the arachnoid mater. The phrenic ($C_5$ branches) and vagus nerves were exposed bilaterally through a dorsal approach. Animals were ventilated on oxygen-enriched air by a positive pressure pump after muscle paralysis with gallamine triethiode (4 mg/kg i.v. initial dose, followed by 4–8 mg/h). The phrenic and vagus nerves were sectioned, desheathed and the central ends of the nerves were mounted on bipolar silver hook electrodes. Small patches of pial membrane were removed from the medullary surface where electrodes were inserted for recording. To provide stability for intracellular and extracellular recording, pneumothorax was established bilaterally and a pressure foot was placed gently on the surface of the medulla over the site of electrode insertion. The spinal cord was exposed from $C_2$ to $C_4$, the dura was cut and an array of four bipolar concentric electrodes were inserted in the reticulospinal tracts bilaterally to identify bulbospinal respiratory neurons by antidromic activation. Exposed nerves were covered with a mixture of vaseline and mineral oil. The spinal cord and other exposed tissue were covered with agar dissolved in Ringer solution. Body temperature was maintained between 37 and 38°C by external heating. Systolic blood pressure was maintained above 90 mmHg when necessary by slow intravenous infusion of *meta*-araminol (100 μg/ml) in glucose/Ringers solution.

### 2.2. Recording and stimulation procedures

Extracellular recordings were obtained from medullary respiratory neurons with either single glass micropipettes or with 3-barrel pipette assemblies for recording and iontophoresis. Recording barrels were filled with 3 M NaCl (5–10 MΩ resistance). Intracelluar recordings were obtained with either single glass micropipettes filled with 3 M KCl (40–70 MΩ) or with compound micropipette assemblies [17] for intracellular recording and extracellular iontophoresis. Iontophoresis barrels were filled with serotonin bitartrate (5HT; 0.04 M, pH 4), 5-MeODMT (0.04 M, pH 4), cinanserin hydrochloride (0.02 M, pH 4), dissolved in bi-distilled water, and methysergide bimaleate (0.005 M, pH 4) dissolved in 165 mM NaCl. Membrane potentials of medullary respiratory neurons were recorded with a DC electrometer equipped with bridge balance and capacity compensation circuits. Extracellularly recorded action potentials were also picked up by the electrometer, amplified (1,000–2,000×) and bandpass filtered (30–3,000 Hz) by an AC preamplifier. Phrenic nerve activity was amplified (5,000–10,000×) and bandpass filtered (100–3,000 Hz). Membrane potentials and action potentials were viewed on an oscilloscope, recorded on a strip chart recorder along with blood pressure, tracheal pressure and end-tidal $CO_2$, and stored on magnetic tape. Action potentials recorded from medullary respiratory neurons and the phrenic nerve were also led off to window-discriminating rate meters and recorded on the strip chart as moving averages (0.2 s time constant) of discharge frequency. Bulbospinal neurons and vagal motoneurons were identified by the presence of antidromically conducted action potentials evoked by stimulating the reticulospinal tracts or vagus nerves.

### 2.3. Iontophoresis

Drugs were injected from iontophoresis barrels with cationic currents delivered from a programmable iontophoresis current generator. Between ejecting periods, drug leakage was prevented by anionic retaining currents (−20 nA). Currents which ejected 5-MeODMT and 5HT were varied until a current intensity was found which produced reproducible 50% inhibition of action potential frequency ($IC_{50}$) as determined from the moving average of discharge frequency.

## 3. Results

### 3.1. Responses of medullary respiratory neurons to iontophoresis of 5-MeODMT and 5HT

Extracellularly recorded responses to iontophoresis of 5-MeODMT and 5HT were obtained from neurons ($n = 39$) in the dorsal (nucleus of the solitary tract) and ventral (n. retroambigualis, n. ambiguus) respiratory groups in 14 experiments. In four additional experiments, intracellularly recorded responses were obtained during iontophoresis from late-discharging expiratory neurons ($n = 4$) located caudal to the obex in the ventral respiratory group. Cells were classified as inspiratory, late inspiratory, post-inspiratory, inspiratory–post-inspiratory and expiratory based on the timing of their discharges in relation to the inspiratory, post-inspiratory and expiratory (silent) phases of phrenic nerve activity. The results obtained with extracellular recording are summarized in Table 1.

Discharges of 28 of 33 cells (85%) recorded extracellularly were depressed by iontophoresis of 5-MeODMT, whereas firing of 5 cells was unaffected. Discharges of 15 of 17 cells (88%) were depressed by 5HT and 2 cells

Table 1
Effects of microelectrophoretically applied 5MeODMT and 5HT on medullary respiratory neurons [a]

| Cell type [c] | $IC_{50}$, 5MeODMT (nA) [b] | $n$ | $IC_{50}$, 5HT (nA) | $n$ |
|---|---|---|---|---|
| (A) Inhibition | | | | |
| DRG-I | 31 (5–60) | 8 | | 0 |
| VRG-IPI | 40 (30–50) | 3 | 40 (30– 50) | 3 |
| VRG-I | 43 (5–70) | 13 | 60 (30–100) | 7 |
| VRG-PI | 45 | 1 | 40 (30– 50) | 4 |
| VRG-E | 65 | 1 | | 0 |
| VRG-LI | 70 (60–80) | 2 | 30 | 1 |
| (B) No response | | | | |
| VRG-I | 10– 70 nA | 3 | 50–70 nA | 2 |
| DRG-I | 50–170 nA | 2 | | |

[a] Extracellular recording.
[b] $IC_{50}$, current necessary to produce 50% depression of peak unitary action potential frequency; mean values, range in parentheses.
[c] Abbreviations: DRG, dorsal respiratory group; VRG, ventral respiratory group; I, inspiratory; IPI, inspiratory–post-inspiratory; PI, post-inspiratory; E, expiratory; LI, late inspiratory.

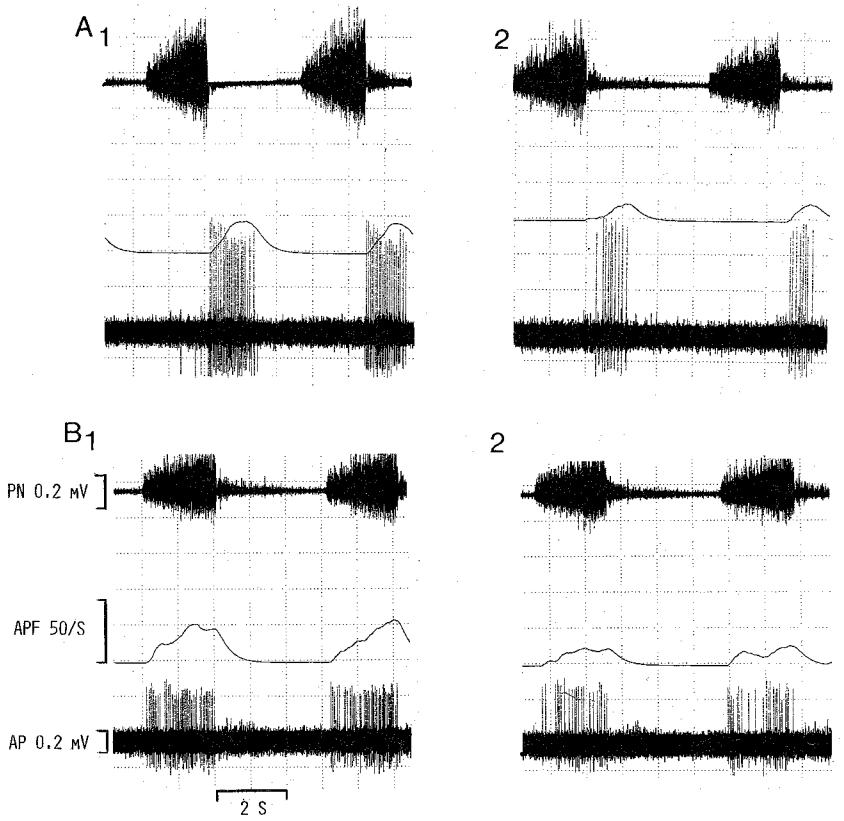


Fig. 1. Reduction of inspiratory and post-inspiratory cell discharges during iontophoretic application of 5-MeODMT. Parts $A_1$–$B_2$ show phrenic nerve activity (PN), action potentials (AP) recorded extracellularly from a post-inspiratory neuron ($A_1$, $A_2$) and from an inspiratory neuron ($B_1$, $B_2$) and moving averages of cell discharge frequency (APF; action potentials per s). Control discharges are seen in $A_1$ and $B_1$. Reduction of cell discharges during iontophoresis (50 nA) of 5-MeODMT (0.04 M) are seen in $A_2$ and $B_2$.

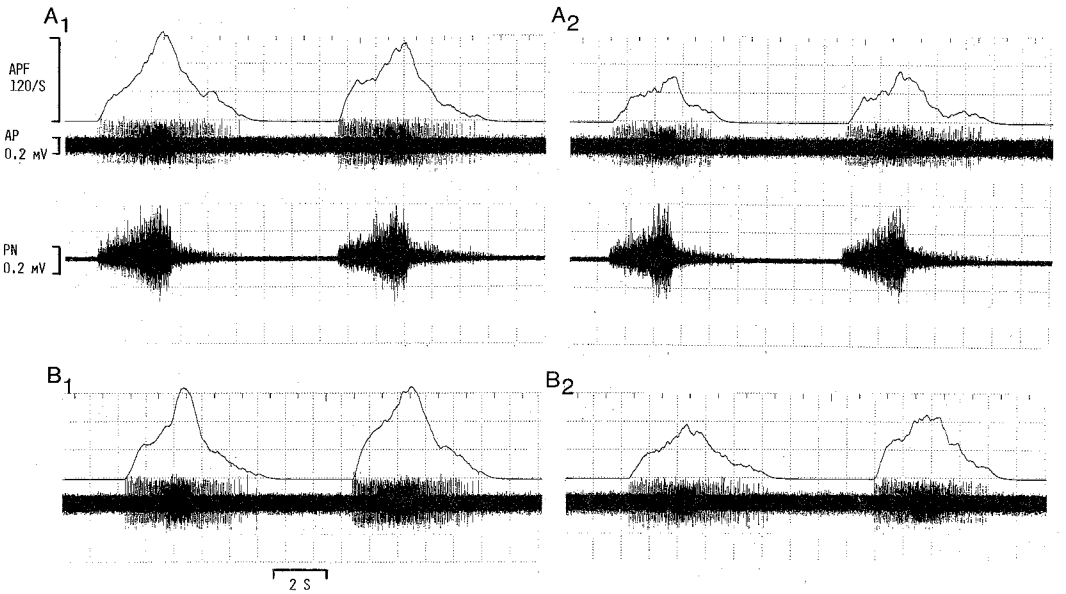


Fig. 2. Reduction of inspiratory–post-inspiratory cell discharges during iontophoresis of 5-MeODMT and 5HT. $A_1$–$B_2$ show action potentials (AP) recorded from a ventral respiratory group (VRG) neuron which discharged during the inspiratory and post-inspiratory phases of phrenic nerve activity (PN). APF, moving average of cell discharge frequency. Control responses are shown in $A_1$ and $B_1$. Cell discharges were reduced by iontophoresis of 5-MeODMT (40 nA, 0.04 M) in $A_2$ and 5HT (40 nA, 0.04 M) in $B_2$.

were unresponsive. Ejecting currents required to produce 50% inhibition ($IC_{50}$) of peak discharge frequency ranged from 5 nA to 80 nA for 5-MeODMT and from 30 nA to 100 nA for 5HT. Fifty percent inhibition occurred after 30–90 s of 5-MeODMT or 5HT application, and recovery to control after the end of the ejecting period occurred over a similar time-course.

Inhibition of the firing of inspiratory, post-inspiratory and inspiratory–post-inspiratory cells of the ventral respiratory group are shown in Figs. 1 and 2.

Of the cells which were depressed by 5-MeODMT and 5HT, 12 were bulbospinal inspiratory neurons (5 in the dorsal respiratory group, 7 in the ventral group) while the remaining 27 cells failed to respond to stimulation of vagus nerve or reticulospinal tracts.

Iontophoretic application of the 5HT-II/1 c receptor antagonists, cinanserin ($n = 10$ cells) or methysergide ($n = 8$), to inspiratory neurons of the dorsal and ventral respiratory groups failed to antagonize the depression of cell firing produced by 5-MeODMT and 5HT when the antagonists were ejected with currents

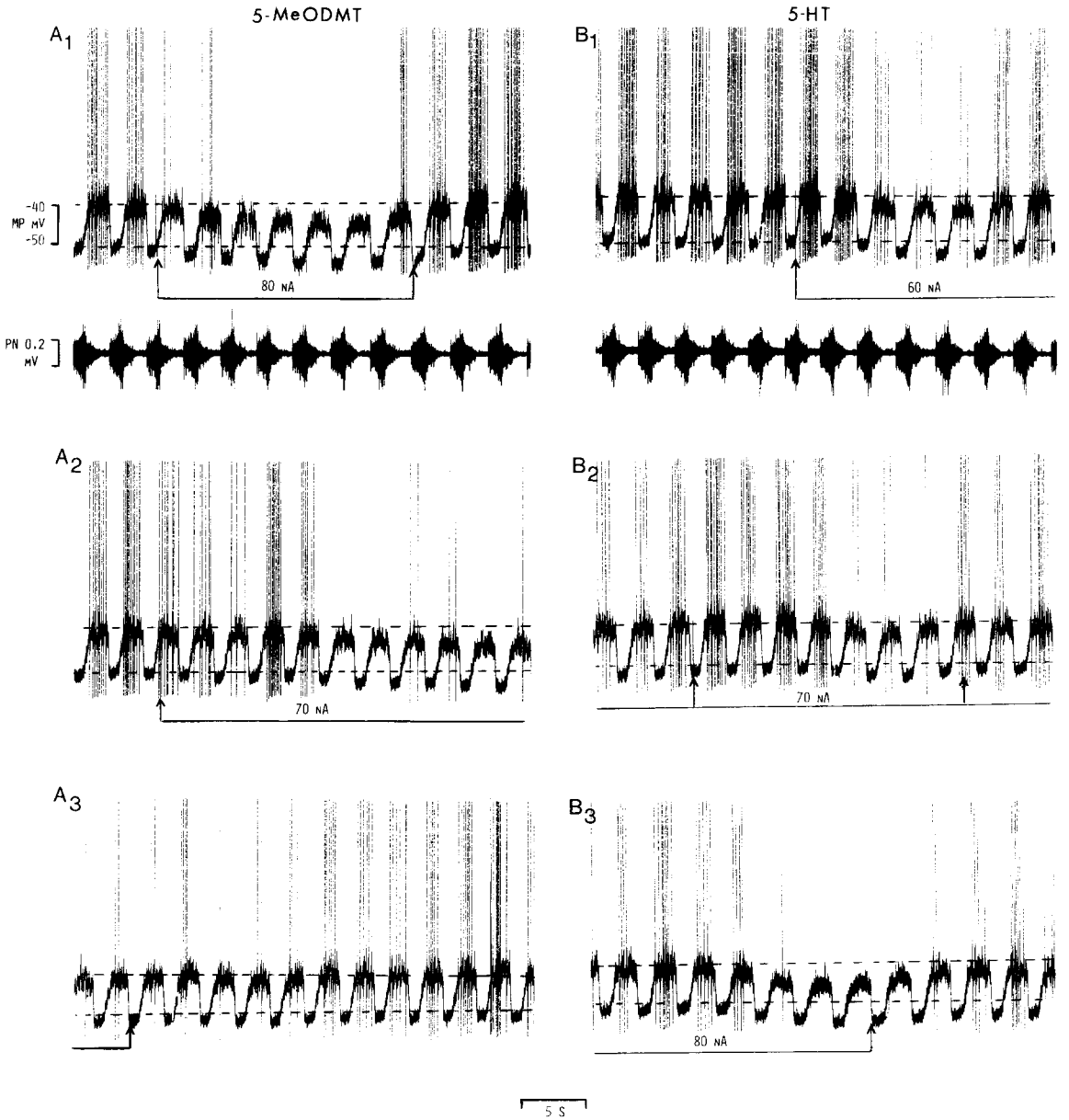


Fig. 3. Hyperpolarization and reduction of discharges in a late-discharging expiratory neuron of the caudal VRG during iontophoresis of 5-MeODMT and 5HT. $A_1$–$B_3$ show membrane potential (MP) of the expiratory neuron and phrenic nerve activity (PN). Dashed lines are references for control inspiratory phased MP (lower lines) and threshold for cell firing (upper lines). $A_1$–$A_3$ and $B_1$–$B_3$ show hyperpolarization of MP and reduction of cell discharges during iontophoresis of 5-MeODMT and 5HT. Ejecting currents and their times of application are indicated by brackets and arrows under records of MP. Middle and bottom traces in A and B are contiguous.

of 20–50 nA. Ejection of the antagonists with currents greater than 50 nA depressed cell firing and reduced spike amplitude, indicative of direct depressant effects.

Intracellular recording from 4 late-discharging expiratory neurons revealed hyperpolarization of the membrane potential and depression of cell firing during iontophoresis of 5-MeODMT or 5HT (Fig. 3).

The degree of inhibition was directly related to the size of the ejecting current. Recovery occurred 30–180 s after the end of ejection.

### 3.2. Responses to intravenous injection of 5-MeODMT

Several types of medullary respiratory neurons were tested for their responsiveness to intravenous doses of 5-MeODMT in 40 experiments. Two types of responses were observed. The first type of response was observed in all experiments after injecting 25–75 μg/kg of 5-MeODMT (43 ± 8.9 μg/kg, mean ± S.E.M.) and consisted of a severe depression of neuronal action potential frequency and phrenic nerve activity. The second type consisted of increased discharge frequency recorded from inspiratory and expiratory neurons and phrenic nerve in 13 of 20 experiments after injection of 10–40 μg/kg of 5-MeODMT (27 ± 2.7 μg/kg). The two responses are dose-dependent since the second (lower dose) response was always converted to the first by additional doses of 5-MeODMT.

### 3.3. Depression of cellular discharges by 5-MeODMT

Intravenous doses of 5-MeODMT (43 ± 8.9 μg/kg) not only reduced phrenic nerve activity and depressed discharges of medullary respiratory neurons but also lowered blood pressure. The effects of 5-MeODMT on phrenic nerve activity, vasomotor sympathetic neural discharges and blood pressure have been described in detail in an earlier report [16]. Although hypotension is not the cause of respiratory depression [16] it did reduce stability required for intracellular recording. Consequently, most responses to the larger doses of 5-MeODMT were recorded with extracellular electrodes. Sixty neurons were recorded from: inspiratory neurons of the dorsal and ventral respiratory group; post-inspiratory, inspiratory–post-inspiratory, late inspiratory neurons and antidromically activated vagal motoneurons of the ventral respiratory group rostral to the obex, and late-discharging expiratory neurons caudal to the obex. All of the cells, irrespective of discharge pattern, exhibited reductions of action potential frequency after injection of the higher doses of 5-MeODMT. The results are summarized in Table 2A.

Action potential frequency of fourteen DRG and twenty-one VRG inspiratory neurons were depressed by 5-MeODMT. All of these cells exhibited action potentials which began with the onset of phrenic nerve activity, increased in frequency to reach a maximum during mid-inspiration and ended abruptly with the termination of inspiratory discharge of phrenic nerve activity. Eight of the DRG neurons and eleven of the VRG neurons were bulbospinal, whereas none were antidromically activated by vagus nerve stimulation. Depression of a DRG inspiratory neuron by 5-MeODMT is illustrated in Fig. 4.

Peak discharge frequency was reduced by 75% within 1 min after injection of 5-MeODMT, 40 μg/kg i.v. (Fig. 4B), along with a similar level of depression of phrenic nerve activity (not shown). Full recovery of both types of discharge occurred after 12 min. After an additional 30 min cinanserin (4 mg/kg i.v.) was injected. Subsequent injection of 5-MeODMT, 40 μg/kg, then produced relatively weak (12%) suppression of neuronal discharge frequency (Fig. 4C) and phrenic nerve activity. Antagonism by cinanserin was evident from similar tests on four additional DRG inspiratory neurons. Other tests were unsuccessful due to loss of recording stability related to bradycardia and hypotension produced by cinanserin.

Table 2
Effects of intravenous doses of 5-MeODMT on medullary respiratory neurons

| | (A) Decreased action potential frequency | | | (B) Increased burst frequency | | |
|---|---|---|---|---|---|---|
| Cell type | *n* | Dose (μg/kg) [b] | % Decrease [c] | *n* | Dose | % Increase [d] |
| DRG-I | 14 | 50 (25–75) | 61.9 (30–100) | | | |
| VRG-I | 21 | 46.4 (25–75) | 68.6 (33–100) | 4 | 18.8 (10–30) | 35 (10–60) |
| VRG-PI | 6 | 50.8 (25–75) | 52 (20–85) | | | |
| VRG-IPI | 6 | 53 (40–60) | 49.1 (30–60) | | | |
| VRG-E | 7 | 39.4 (25–70) | 65.7 (50–100) | 20 | 20.5 (10–40) | 36.5 (8–100) |
| VRG-LI | 2 | 25, 32 | 60, 75 | | | |
| VMN-I [a] | 4 | 46.3 (25–70) | 51.3 (49–56) | | | |

[a] VMN-I, vagal inspiratory motoneuron. See Table 1 for other abbreviations.
[b] Mean (range).
[c] Percent decrease from peak frequency of action potentials within a burst. Peak frequency was obtained by measuring peaks of rate meter records. Average values were calculated from 10 consecutive discharges before and after injection of 5-MeODMT.
[d] Percent increase obtained by measuring time required for 10 inspiratory- or expiratory-phased bursts of action potentials before and after 5-MeODMT.

Discharges of neurons which fired during the post-inspiratory phase of phrenic nerve activity (6 post-inspiratory neurons, 6 inspiratory–post-inspiratory neurons) were depressed by 5-MeODMT. Fig. 5 illustrates the typical depressant effect of 5-MeODMT on post-inspiratory neurons.

Post-inspiratory neurons discharge with the onset of the post-inspiratory after discharge of phrenic nerve activity (Fig. 5A) and cease firing during the expiratory silent period [36,37]. Doses of 5-MeODMT which depressed phrenic nerve activity also depressed the spike frequency of post-inspiratory neurons without altering their discharge pattern or their temporal occurrence with respect to phrenic nerve activity (Fig. 5B). Inspiratory–post-inspiratory neurons, on the other hand, exhibited a continuous spike discharge of much lower frequency after injection of doses of 5-MeODMT which depressed phrenic nerve discharges. None of the neurons were antidromically activated by stimulation of vagus nerves or reticulospinal tracts.

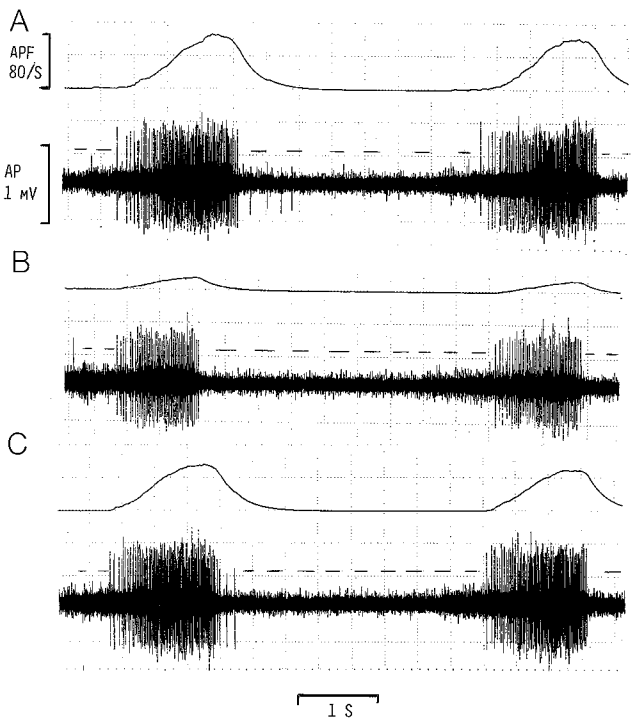


Fig. 4. Effects of intravenous administration of 5-MeODMT and the 5HT receptor antagonist cinanserin on discharges of a bulbospinal inspiratory neuron of the dorsal respiratory group (DRG). A–C show extracellularly recorded action potentials (AP) and the moving average of cell discharge frequency (APF; action potentials per s). Dashed lines through AP indicates the voltage detection level of the window discriminator for spike counting. A: activity recorded before drug injection. B: reduction of APF recorded 3 min after i.v. injection of 5-MeODMT, 40 $\mu$g/kg. C: antagonism by cinanserin of the response to 5-MeODMT. Thirty minutes after recovery from the response to 5-MeODMT shown in B, cinanserin (4 mg/kg, i.v.) and then 5-MeODMT (40 $\mu$g/kg i.v.) were injected. Records in C were taken 10 min after administration of 5-MeODMT. In the absence of cinanserin, 5-MeODMT produced a 75% reduction of APF (B), whereas after cinanserin an identical dose of 5-MeODMT produced 12% reduction (C).

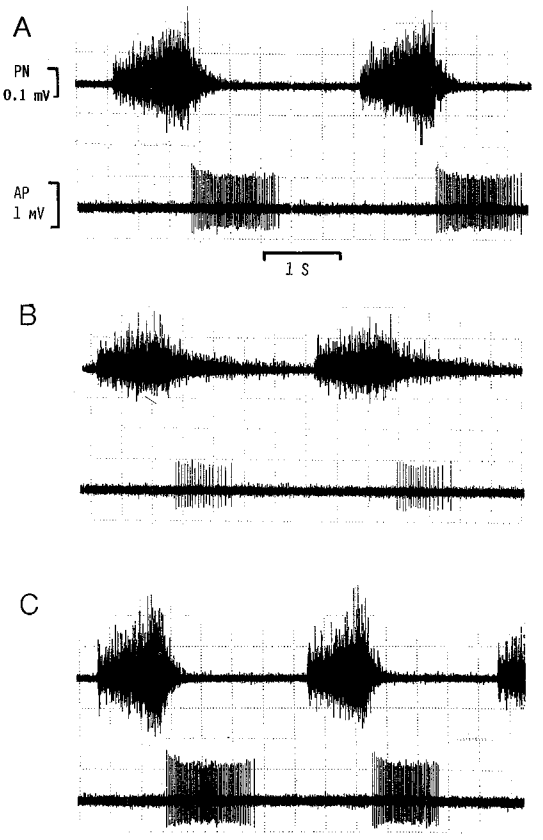


Fig. 5. Reduction of post-inspiratory cell discharges by i.v. injection of 5-MeODMT. Traces show phrenic nerve activity (PN) and action potentials recorded extracellularly (AP) from a VRG neuron which discharged with the post-inspiratory discharge of PN. A: control discharges. B: reduction in the number of cell action potentials recorded 3 min after injection of 5-MeODMT, 40 $\mu$g/kg. Reduction in AP amplitude probably reflects movement of brain tissue caused by arterial hypotension. C: recovery of discharges 30 min after injection.

Late-discharging expiratory neurons ($n = 7$) exhibited hyperpolarization and reduction of cell firing after injection of 5-MeODMT, as shown in Fig. 6. Furthermore, the delay of discharge onset relative to the phrenic nerve discharge was increased. In this case, but not in others, the greater hyperpolarization of membrane potential (13 mV) produced by 5-MeODMT (Fig. 6B) resulted in reversal of the inspiratory-phased waves of hyperpolarization.

5-MeODMT also depressed the spike frequency of two late inspiratory neurons and 4 antidromically activated vagal inspiratory motoneurons which were recorded extracellularly.

### *3.4. Tachypneic responses to 5-MeODMT*

The frequency of phrenic nerve discharges increased within 60–90 s following i.v. injection of 5-MeODMT

(27 ± 2.7 μg/kg). The tachypnea was accompanied by shorter inspiratory discharges and expiratory silent periods, whereas changes in the durations of post-inspiratory discharges were not consistent. The duration of post-inspiratory discharges was decreased in four experiments, lengthened in five and unchanged in four. Peak action potential frequency during the inspiratory phase was unchanged in 11 experiments and reduced by less than 10% in 2 others. Peak action potential frequency of the post-inspiratory after discharge was reduced (39 ± 11.3%) in ten experiments. Doses of 5-MeODMT which produced tachypnea also increased mean arterial pressure by 11.7 ± 3.16 mmHg.

Intracellular recordings were obtained from 24 medullary respiratory neurons during such tachypneic episodes. Responses were recorded from four inspiratory neurons rostral to the obex in the ventral respiratory group (VRG) and from 20 late-discharging expiratory neurons in the VRG caudal to the obex. All neurons under control conditions exhibited maximum membrane potentials from −50 to −70 mV. The response of an inspiratory VRG neuron to 5-MeODMT is shown in Fig. 7.

The membrane potential under control conditions (Fig. 7A) exhibited hyperpolarization during the post-inspiratory phase of phrenic nerve activity which at first declined relatively rapidly, then more gradually during the expiratory phase. After injection of 5-MeODMT (Fig. 7B), the post-inspiratory hyperpolarization was reduced in magnitude and duration, whereas the plateau of hyperpolarization in late expiration was abolished. Consequently, more frequent bursts of action potentials occurred in association with increased discharge frequency of phrenic nerve activity. Similar responses were recorded from three other inspiratory VRG neurons.

Late-discharging expiratory neurons of the caudal medulla also responded to the lower doses of 5-MeODMT with increased discharge frequency, as seen in Fig. 8.

The membrane potential patterns of caudal VRG expiratory neurons recorded under control conditions

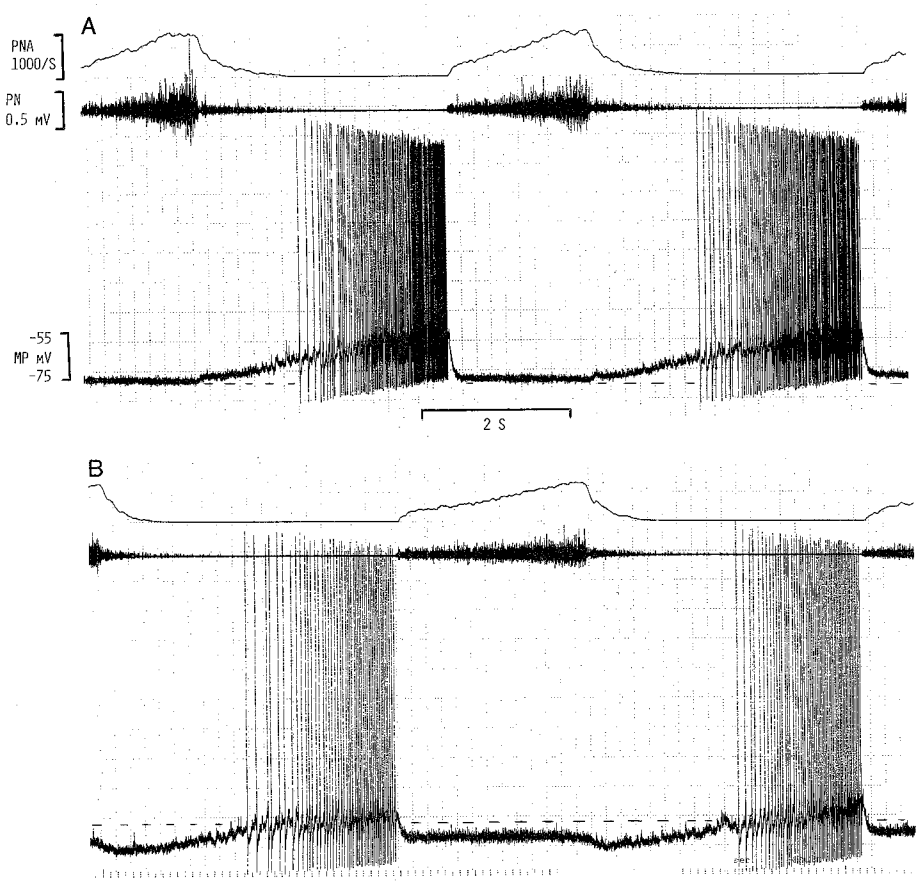


Fig. 6. Hyperpolarization and depressed firing of a late-discharging expiratory neuron of the caudal VRG by i.v. injection of 5-MeODMT. Traces in A and B show the membrane potential (MP) of the expiratory neuron, phrenic nerve activity (PN) and the frequency (moving average) of phrenic nerve action potentials (PNA). A: control discharges. B: records taken 3 min after i.v. injection of 5-MeODMT, 25 μg/kg. Dashed lines refer to maximum inspiratory-phased MP (−75 mV) recorded before 5-MeODMT. In response to 5-MeODMT, the MP hyperpolarized by 13 mV, the cell discharged at a lower frequency and the delay of discharge onset from the phrenic nerve discharge was increased.

were similar to those described in detail in other studies [4,11,31,36]. The pattern (Figs. 6A and 8A) consists of a maximal wave of hyperpolarization during the inspiratory phase of phrenic nerve activity which is followed by more gradually declining hyperpolarization during the post-inspiratory phrenic nerve after-discharge until the neuron reaches the threshold for action potential discharge. After injection of 5-MeODMT (Fig. 8B), inspiratory-phased hyperpolarization was greater but also shortened in association with briefer inspiratory bursts of phrenic nerve activity. The post-inspiratory decline of hyperpolarization toward the threshold was more rapid. These temporal changes led to more frequent and earlier discharges of the 20 expiratory neurons.

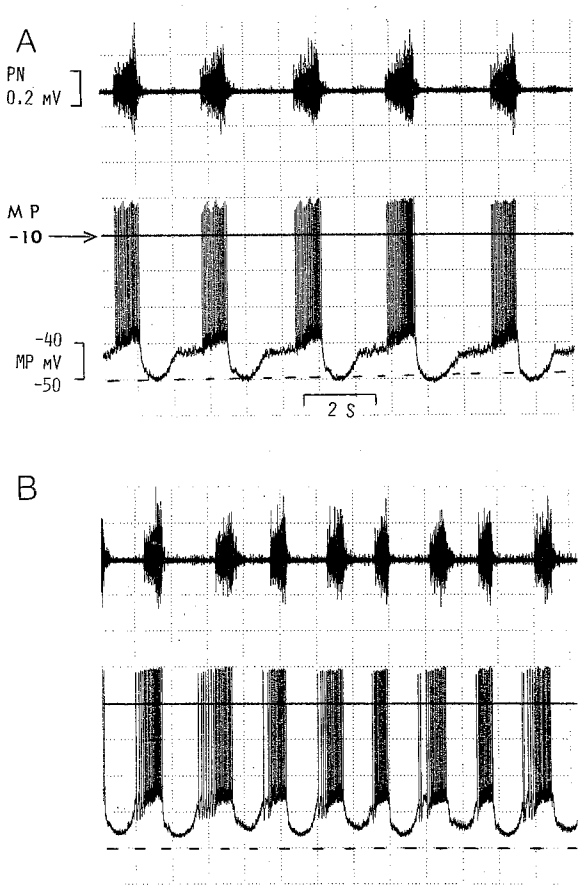


Fig. 7. Increased discharge frequency of a non-antidromically activated inspiratory neuron of the VRG after injection of 5-MeODMT. Records show phrenic nerve activity (PN) and the membrane potential (MP) of the inspiratory neuron. Under control conditions (A), the MP of the cell is maximally hyperpolarized during the post-inspiratory phase of phrenic nerve activity (dashed reference line) and exhibits a later, more gradual decline in MP during the expiratory period until the threshold for discharge is reached with the onset of phrenic nerve discharge. After injection of 5-MeODMT, 15 μg/kg (B), post-inspiratory phased hyperpolarization is reduced in magnitude and duration, the expiratory-phased wave of hyperpolarization is absent and inspiratory discharges of the cell and phrenic nerve are more frequent.

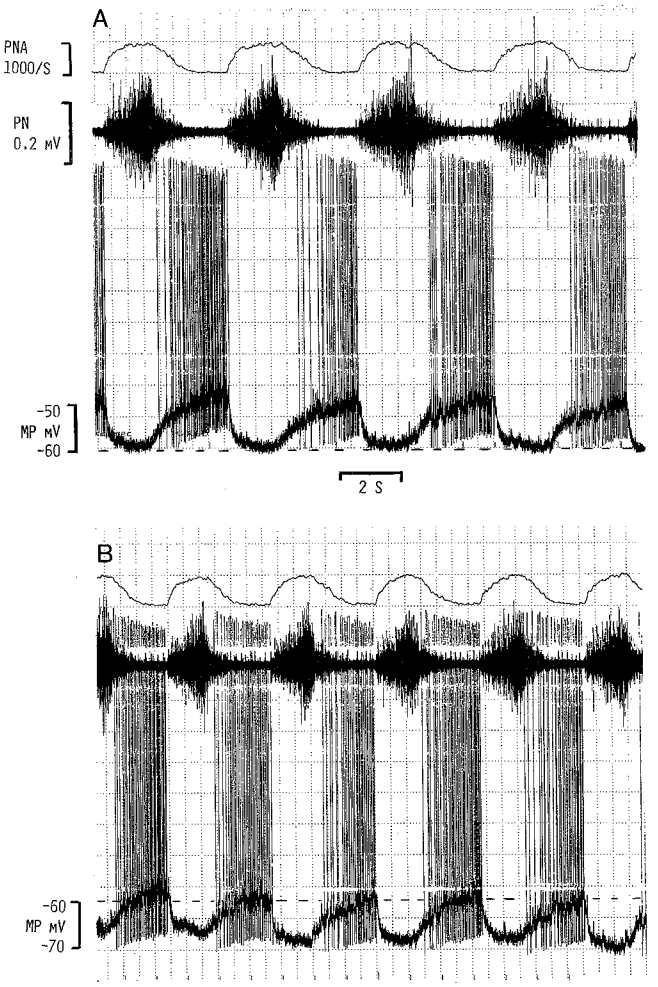


Fig. 8. Increased discharge frequency of a bulbospinal expiratory neuron in the caudal VRG after injection of 5-MeODMT. Records show membrane potential (MP) of the neuron, phrenic nerve activity (PN) and the frequency of action potentials recorded from the phrenic nerve (PNA). Control records are shown in A. After a 15 μg/kg injection of 5-MeODMT (B), the MP is hyperpolarized (dashed reference lines). In addition, the inspiratory-phased waves of hyperpolarization are more brief in association with briefer discharges of PN, and both the cell and PN discharge more frequently.

### 3.5. *Anti-apneustic effects of 5-MeODMT*

Apneusis, characterized by prolonged inspiratory episodes [23], was observed in two experiments. Since pentobarbital can suppress the inspiratory off-switching mechanism [44] it is assumed that the apneusis was related to anesthesia. In one experiment, intravenous injection of 5-MeODMT (15 μg/kg) converted the apneustic phrenic nerve activity to a relatively normal pattern of shorter and more frequent inspiratory discharges. In another experiment, application of 5-

MeODMT to the dorsal surface of the medulla had a similar anti-apneustic effect on phrenic nerve activity and the membrane potential properties of an antidromically identified vagal expiratory motoneuron, as shown in Fig. 9. Under control conditions, prolonged inspiratory discharges of phrenic nerve activity lasting longer than 4 s (Fig. 4A) were recorded. The membrane potential of the vagal expiratory motoneuron declined gradually from a maximum during early inspiration with occasional discharge until robust firing occurred during expiration. Late-discharging expiratory bulbospinal neurons exhibit similar membrane potential patterns during apneusis produced by hypoxia and i.v. injection of NMDA receptor antagonists [9,39]. Three minutes after application of 0.5 ml 5-MeODMT (50 $\mu$M) to the dorsal surface of the medulla (Fig. 9B), the apneustic patterns were converted to shorter, more normal patterns. After 10 min (Fig. 9C), effects of 5-MeODMT resembling those produced by i.v. doses ($43 \pm 8.9$ $\mu$g/kg) of 5-MeODMT were observed. The intensity of phrenic nerve discharges was reduced, the membrane potential of the expiratory neuron was hyperpolarized by 15 mV and the expiratory-phased membrane potential failed to reach threshold for ac-

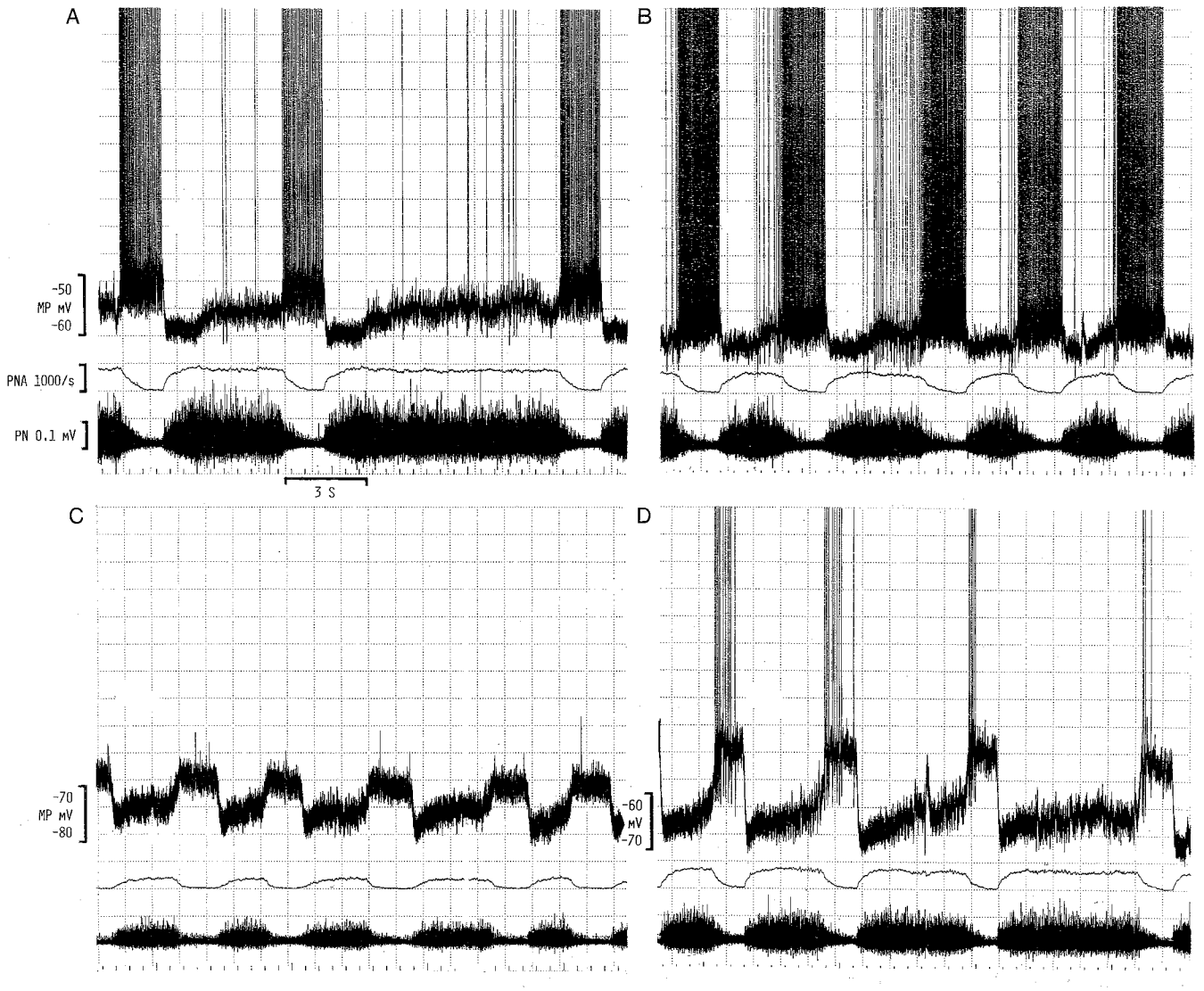


Fig. 9. Shortening of apneustic discharges alter application of 5-MeODMT to the dorsal surface of the medulla. Records show the membrane potential of an expiratory vagal motoneuron (MP), phrenic nerve activity (PN) and frequency of action potentials recorded from the phrenic nerve (PNA). Prolonged inspiratory discharges of phrenic nerve activity (apneusis) are seen in A. The neuron gradually depolarized and discharged occasionally during the prolonged discharge of PN and fired more vigorously during expiration. B: records taken 3 min after applying 5-MeODMT (0.5 ml, 50 $\mu$M, pH 7.0, in Ringer solution) to the surface of the medulla. Apneustic PN discharges were shortened and inspiratory and expiratory discharges were more frequent. C: records taken 10 min after 5-MeODMT show hyperpolarization, absence of expiratory cell discharge and reduced intensity of PN. After 25 s of asphyxia (D) expiratory discharges were reinstated and PN was more intense.

tion potential production. When, however, asphyxia was induced by turning off the ventilatory pump for 25 s, phrenic nerve discharges become more intense and the expiratory neuron commenced firing. Earlier studies [16,28] demonstrated that inhibition of phrenic nerve activity by systemic injection of 5-MeODMT or the 5HT precursor 5-hydroxytryptophen is removed during stimulation of peripheral and central nervous system chemoreceptors. The reversal of inhibition can be attributed to chemoreceptor-activated firing of medullary respiratory neurons [19,39]. In the experiment illustrated in Fig. 9, apneustic discharges returned 5 min after washout of 5-MeODMT with topical application of Ringer solution. Subsequent i.v. injection of the same volume (0.5 ml) of 50 $\mu$M 5-MeODMT (1 $\mu$g/kg) had no effect on respiratory activity, however, antiapneustic effects were produced by a 15 $\mu$g/kg i.v. dose. It is assumed, therefore, that the effects seen in Fig. 9B,C are attributable to actions on brainstem respiratory neurons.

## 4. Discussion

Intravenous administration of 5-MeODMT produced different dose-dependent effects on discharges of medullary respiratory neurons and phrenic nerve activity. Doses ranging from 25 to 75 $\mu$g/kg depressed respiratory discharges. These results represent a combination of presynaptic actions, since there is extensive synaptic interaction among respiratory neurons [15,19,36,38], and post-synaptic effects, since depression is also produced by iontophoresis of 5-MeODMT. 5-MeODMT has a relatively high affinity for 5HT-1A receptors [10], and other agonists with more selective affinity for these receptors, such as 8-OHDPAT [3], hyperpolarize and decrease the excitability of respiratory [17,18] and non-respiratory [2,3] neurons. Furthermore, in the iontophoresis experiments of the present study, cinanserin and methysergide failed to antagonize the suppression of discharges by 5-MeODMT. These antagonists, which have a relatively high affinity for 5HT-II/1c receptors [10], block excitatory but not inhibitory responses of neurons to iontophoresis of 5HT [1,40]. Therefore, a post-synaptic component of the inhibition appears to be due to activation of 5HT-1A receptors. Additional mechanisms, however, seem to be involved. Inhibition of discharges produced by the larger doses of 5-MeODMT was reduced by i.v. injection of cinanserin. Earlier studies also showed that suppression of phrenic nerve discharges [16] and breathing [24] by systemic administration of 5-MeODMT are reduced by cinanserin and methysergide. These findings point to one of several possible presynaptic mechanisms. By binding to 5HT-II/1 c receptors on non-serotoninergic inhibitory neurons, 5-MeODMT could increase cell firing and the release of a neurotransmitter which adds to the post-synaptic inhibition mediated by 5HT-1A receptor activation. Intravenous administration of cinanserin would reduce the inhibition by blocking 5HT-II/1 C receptors.

5HT-1A receptors are present on serotoninergic neurons of the medullary raphe complex. These neurons, which are presumed to be the major source of serotonin-containing boutons found in close apposition to respiratory neurons of the medulla and spinal cord [12–14,21,43], are depressed by iontophoretic application of 8OHDPAT, 5-MeODMT and 5HT [26,27]. Feedback inhibition mediated by activation of 5HT-1A autoreceptors explains why i.v. administration of 5-MeODMT depresses sympathetic vasomotor discharges [26,27] and urinary bladder reflexes [41]. Such a mechanism does not, however, adequately explain the depression of respiratory neural discharges by 5-MeODMT, since it implies that 5HT has a predominantly excitatory effect on respiratory neurons, contrary to what was observed in the present and previous [5,8,24,28] investigations. Furthermore, chemical destruction of serotoninergic neurons or depletion of 5HT stimulates breathing [35] and these treatments do not prevent depression of phrenic nerve discharges by 5-MeODMT [16,24].

The effect produced by 10–40 $\mu$g/kg doses of 5-MeODMT consisted of increases in discharge frequency of inspiratory and expiratory neurons. In addition, expiratory neurons discharged earlier with respect to phrenic nerve activity. Increases in discharge frequency of inspiratory and expiratory neurons are also produced by i.v. injection of 8-OHDPAT [17,18], therefore the effect seems to be attributable to activation of 5HT-1A receptors. The present experiments provide no direct evidence regarding which types of respiratory neurons are directly involved in the alteration of respiratory rhythm. A candidate, however, would be the early inspiratory neurons of the medulla. These neurons are thought to synaptically inhibit and consequently delay the firing of late inspiratory and post-inspiratory neurons which terminate inspiration [15,38]. The discharge of early inspiratory neurons is initially robust but declines under the influence of synaptic inhibition [15,22,36,38] and, possibly, an activity-dependent calcium-activated potassium conductance [29,30]. Augmentation of the intrinsic conductance through activation of a 5HT-1A receptor-mediated increase in potassium conductance [2,3] could result in briefer early inspiratory discharges, hence earlier release of late inspiratory and post-inspiratory neurons from synaptic inhibition. The end effects would be shorter inspiratory discharges, earlier firing of expiratory neurons and therefore, increased discharge frequency of inspiratory and expiratory neurons.

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