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SYNAPTIC INTERACTION BETWEEN MEDULLARY RESPIRATORY NEURONES DURING APNEUSIS INDUCED BY NMDA-RECEPTOR BLOCKADE IN CAT

BY JACK L. FELDMAN*, UWE WINDHORST, KLAUS ANDERS AND DIETHELM W. RICHTER

From the University of Göttingen, Centre of Physiology and Pathophysiology, Humboldtallee 23, 3400 Göttingen, FRG

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SUMMARY

1. Termination of inspiration is an essential component of respiratory rhythm generation and its perturbation can result in apneusis, i.e. significant prolongation of inspiratory activity. In an effort to further analyse inspiratory termination mechanisms, we studied the postsynaptic events in respiratory neurones during apneustic respiratory periods, and compared them to normal respiratory cycles.

2. Experiments were performed in pentobarbitone-anaesthetized, paralysed, thoracotomized cats ventilated with a constant volume or a cycle-triggered constant pressure pump. Apneusis, separated by normal cycles, was induced as follows: the animal was ventilated by a cycle-triggered pump that normally inflated the lungs during the inspiratory burst of phrenic nerve discharge. The NMDA-receptor blocker MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-iminemaleate] (0·3–0·7 mg/kg) was administered intravenously, and, for designated breaths, inflation of the lungs was withheld during neural inspiration.

3. Membrane potential trajectories of forty-one late expiratory (E-2) and eight postinspiratory (PI) neurones of the caudal ventral respiratory group were analysed before and/or after MK-801 administration, during normal and apneustic periods.

4. Before MK-801 administration, withholding lung inflation caused modest (10–20%) lengthening of the inspiratory period; after MK-801 administration, withholding inflation caused apneusis. Provided that the lungs were inflated during the inspiratory phase, the temporal pattern of phrenic nerve, recurrent laryngeal nerve and membrane potential trajectories of E-2 and PI neurones were not significantly altered by MK-801. Apneusis following NMDA-receptor blockade produced consistent changes in the synaptic activation patterns of E-2 neurones. In particular, the slow late inspiratory-related depolarization pattern of E-2 neurones was consistently retarded during apneustic inspiratory phases when compared to normal inspiratory phases. This was due to continuation of Cl^--mediated synaptic inhibition of E-2 neurones. Superior laryngeal nerve stimulation stopped apneusis and sustained membrane hyperpolarization of E-2 neurones similar to lung inflation.

* Permanent address and address for correspondence: Systems Neurobiology Laboratory, Department of Kinesiology, UCLA, 405 Hilgard Avenue, Los Angeles, CA 90024-1527, USA.

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5. During the plateau phase of apneusis, correlated 10–20 Hz oscillations could be observed in the integrated phrenic and recurrent laryngeal nerve activities as well as in the membrane potential of E-2 neurones.

6. We conclude that: (i) the prolonged inhibition of E-2 neurones during apneusis is indicative of the process responsible for the prolongation of the inspiratory phase. (ii) Synaptic interactions between medullary respiratory neurones and spinal respiratory motoneurones producing burst patterns of motor output do not require NMDA-receptor-mediated pathways. (iii) Pathways used by pulmonary and laryngeal afferents to terminate inspiration also do not require NMDA-receptor-mediated function. (iv) NMDA-receptor controlled pathways necessary for inspiratory termination seem to be activated in the absence of lung inflation and to involve the pontine respiratory group of neurones. (v) MK-801-induced apneusis affects the interaction between early-inspiratory and late-inspiratory neurones resulting in oscillations.

INTRODUCTION

The orderly transition from one respiratory phase to the next is basic to the rhythm of breathing. The transition marking the end of inspiration has been particularly intensively studied (q.v. Feldman, 1986). Normally, the inspiratory motor activity which increases slowly to expand the thorax is terminated rapidly, and followed by a lower level of declining activity during the postinspiratory period (Remmers, Baker & Younes, 1979). Both peripheral afferent signals and brainstem neuronal activity contribute to the mechanisms that determine the timing of inspiratory termination. Breuer (1868) observed in rabbits that increases in lung volume decrease inspiratory duration, and vice versa. Subsequently, the pulmonary stretch receptor-mediated inspiration-shortening reflex has been well characterized in many mammalian species, including cats (Clark & von Euler, 1972; Feldman & Gautier, 1976), rats (Bonham & McCrimmon, 1990), rabbits (Karczewski, Naslonska & Romaniuk, 1980) and man (Clark & von Euler, 1972; Gautier, Bonora & Gaudy, 1981). In lightly anaesthetized or decerebrate mammals, the absence of phasic activation of pulmonary stretch receptor afferents prolongs inspiratory duration, but the appearance of the phase transition itself appears normal. The effects of lung inflation on the impulse activity of brainstem inspiratory and expiratory neurones has been well documented (q.v. Feldman, 1986; Ezure, 1990). Also the subthreshold effects of lung inflation on the membrane potential of second-order afferent neurones (Camerer, Richter, Röhrig & Meesmann, 1979; Anders & Richter, 1987; Anders, Ohndorf, Dermietzel & Richter, 1990) have been described, but there is little information about the lung stretch receptor-mediated inhibition of inspiratory neurones and activation of expiratory neurones. The first goal of this study was to compare the pattern of postsynaptic activity in medullary expiratory neurones in cycles with and without pulmonary stretch receptor activity. We chose to study expiratory neurones with augmenting discharge patterns (late expiratory (E-2) neurones) located in the caudal ventral respiratory group. These E-2 neurones are of interest because their postsynaptic activity reflects much of the activity in the brainstem respiratory network (Richter, Ballantyne & Mifflin, 1985; Ballantyne & Richter, 1986, Anders, Ballantyne, Bischoff, Lalley & Richter, 1991). Another

important group of respiratory neurones, those which discharge during the postinspiratory phase, are found scattered amongst the E-2 neurones; we studied them during occasional penetrations.

Small lesions in the dorsolateral rostral pons in anaesthetized or decerebrate cats, in the region of the medial and lateral parabrachial and Kölliker–Fuse nuclei (pontine respiratory group; Bertrand & Hugelin, 1971; von Euler, Marttila, Remmers & Trippenbach, 1976; Feldman & Gautier, 1976) result in a profound alteration of inspiratory termination in the absence of pulmonary stretch receptor activity. Thus, when signals related to lung inflation are withdrawn, inspiratory activity, which begins normally, remains at a plateau level (for hours if blood gas homeostasis is otherwise maintained). This pattern is referred to as 'apneusis' (Stella, 1938). Apneusis in the absence of pulmonary stretch receptor activity can also be produced by pharmacological means, such as following doses of barbiturate necessary to produce very deep anaesthesia (Feldman & Gautier, 1976; Younes, Remmers & Baker, 1978). Recently, Foutz, Champagnat and Denavit-Saubié (1988) have shown that systemic administration of a potent non-competitive blocker of *N*-methyl-D-aspartic acid (NMDA) receptors, MK-801, can produce apneusis similar to that produced by lesions in the pontine respiratory group. We chose this method to induce apneusis. The second goal of this study was to determine the changes induced in the medullary respiratory network, as reflected in E-2 and postinspiratory (PI) neurone activity, during apneusis. Since laryngeal afferents may normally quench or suppress inspiratory activity (Iscoe, Feldman & Cohen, 1979; Richter, Ballantyne & Remmers, 1987); a third goal was to see whether this still occurred during the apneusis produced by MK-801.

Reports have been given in abstract form (Feldman, Windhorst, Anders & Richter, 1990*b*; Windhorst, Feldman, Anders & Richter, 1990).

METHODS

Anaesthesia and drugs

Successful experiments were performed on four adult (3·3–3·6 kg) anaesthetized cats. Anaesthesia was initiated by intraperitoneal injection of sodium pentobarbitone (40 mg/kg). Subsequent smaller doses (~ 4–8 mg) were administered intravenously whenever phrenic nerve activity, respiratory frequency and/or blood pressure increased spontaneously or in response to nociceptive test stimuli. Atropine sulphate (0·1–0·2 mg/kg I.V.) was given to reduce salivation, and dexamethasone (0·2 mg/kg, I.M.) was administered as a prophylaxis against brain edema. Prior to recording respiratory neurones, animals were paralysed with gallamine triethiodide (~ 12–15 mg/kg I.V. initially and then 1–2 mg/kg hourly) and mechanically ventilated by a positive pressure pump with oxygen-enriched air (see below). In some experiments, the arterial blood pressure was stabilized by infusing saline solution containing adrenaline (40 mg/ml) and glucose (27 mg/ml).

Surgical preparation

Femoral veins were catheterized for injection of fluids and drugs, and a femoral artery was cannulated for measurement of blood pressure. The trachea was cannulated for artificial ventilation and measurement of tracheal pressure. After mounting the animal's head rigidly in a stereotaxic headholder, the T1 and L5 vertebral processes were exposed and clamped to suspend the thorax and abdomen. The phrenic, recurrent and superior laryngeal nerves were dissected by a dorsal approach. The phrenic and recurrent laryngeal nerves were cut peripherally, desheathed and placed on bipolar silver wires for recording. The superior laryngeal nerve was left intact and prepared for stimulation. The spinal cord was exposed by laminectomy from C2 to C4 for

stimulating the reticulospinal tracts of both sides with an array of four bipolar concentric steel electrodes with tip diameters of 100 μm. After placement of these electrodes, the spinal cord was covered with agar dissolved in Ringer solution. A wide pneumothorax was established bilaterally to avoid respiratory movements of the thorax and to increase the stability for intracellular recording. Body temperature was maintained between 36 and 38 °C by external heating.

An occipital craniotomy was performed to expose the caudal brainstem. The dura was carefully cut, and the arachnoid membrane removed. Just prior to recording, a small patch of the medullary surface was cleared of pia to allow insertion of electrodes. A horseshoe-shaped pressure foot was placed gently on this area to increase stability of intracellular recordings.

Recording and stimulation procedures

The superior laryngeal nerve was stimulated with brief bursts of electrical pulses (width 50 μs; rates 5–50 Hz; voltage 0·5–1 V). The spinal cord was stimulated using single pulses of 30–70 V for 100–150 μs.

Nerve signals from phrenic and recurrent laryngeal nerves were amplified ($\sim$ 2000–10000 ×), bandpass filtered (0·1–3 kHz), displayed on an oscilloscope and a chart recorder as a direct discharge or as moving averages (low-pass filtered; $\tau \approx$ 10–100 ms). Phrenic nerve activity was taken as an index of the central respiratory rhythm.

E-2 and PI neurones were localized in the ventral respiratory group caudal to the obex. Neurones were recorded intracellularly with glass pipettes with long thin shanks filled with 3 M-KCl (resistance 30–70 MΩ). They were connected via an Ag–AgCl wire to an intracellular amplifier (Axoclamp-2A; frequency response: (DC, 5 kHz)). Stable penetrations were typically obtained following positive current pulses or controlled capacitative 'ringing'. Membrane potentials were recorded in DC mode, amplified and displayed on an oscilloscope and a chart recorder.

Signals from nerve and intracellular recordings (DC coupled, low and high gain), as well as arterial blood and tracheal pressures, and trigger pulses were displayed on-line on a thermal chart recorder (Gould TA 2000) and were recorded on a wideband FM tape recorder (Racal: V-Store; frequency response: (DC, 5 kHZ)) for off-line examination and analysis.

Artificial ventilation

After paralysis, cats were artificially ventilated by a constant volume pump (Harvard). Ventilatory volume and rate were adjusted to maintain an end-tidal CO_2 level of 3–5 % vol. The O_2 partial pressure of the inspiratory gas and the end-tidal CO_2 pressure were continuously monitored with CO_2- and O_2-meters. Typically, at the time the population of expiratory neurones was localized, ventilation was switched to a constant pressure cycle-triggered pump (Feldman & Gautier, 1976) that worked as follows: phrenic nerve activity was low-pass filtered (RC circuits, $\tau \sim$ 100 ms). An electronic threshold detector was adjusted to produce a square-wave pulse during the period from the onset of the inspiratory burst of phrenic nerve activity to its abrupt decline (inspiratory pulse). The inspiratory pulse activated a power transistor, which resulted in the opening of a solenoid valve connected between a constant pressure source of room air ($P \sim$ 10 cmH_2O) and the lung, resulting in lung inflation. When the inspiratory pulse terminated, the valve closed, and the lung vented to atmosphere via a resistive load ($\sim$ 1–2 cmH_2O). Thus, during control cycles, phrenic nerve activity resulted in inflation of the lungs and the resultant activation of pulmonary stretch receptors, with timing similar to an intact animal. Test cycles without phasic pulmonary stretch receptor activity were produced by blocking the solenoid activation during a phrenic nerve burst, resulting in 'non-inflation' of the lung. These tests were performed before and after intravenous administration of 0·3–0·7 mg/kg MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-iminemaleate, Sigma).

RESULTS

Membrane potential trajectories of forty-one E-2 and eight PI neurones were tested for their response to inflation and non-inflation. Fifteen E-2 neurones were tested before and twenty-four after MK-801 (0·3–0·7 mg/kg) was administered intravenously; two E-2 and one PI neurone were studied both before and after MK-801 injection. All E-2 neurones could be antidromically activated from the spinal

cord. PI neurones could not be activated antidromically from the spinal cord, the superior laryngeal nerve or the vagal trunk.

Effects of lung inflation on the respiratory rhythm and neuronal membrane potential prior to MK-801 administration

The basic pattern of nerve activity and membrane potential trajectories of E-2 and PI neurones was similar when comparing cycles with and without lung inflation (Fig. 1). In both cases, the responses were as follows:

(i) phrenic nerve discharge had an abrupt onset marking the beginning of the inspiratory phase, a slowly augmenting phase of activity, and an abrupt decline marking the end of the inspiratory phase, normally followed by an 'after-discharge' during postinspiration;

(ii) recurrent laryngeal nerve discharge had an abrupt onset slightly preceding that of the phrenic nerve, followed by a slow augmentation to a plateau during the inspiratory phase. There was also a burst of declining activity during the postinspiratory phase;

(iii) E-2 neurones were abruptly hyperpolarized (mean $\pm$ S.D., $9{\cdot}8 \pm 4{\cdot}4$ mV; $n = 21$; range 2·4–16 mV) at the beginning and then slowly depolarizing during the last half of inspiration. At the end of the inspiratory phase, there was rapid depolarization (mean $\pm$ S.D., $4{\cdot}4 \pm 2{\cdot}1$ mV; $n = 17$; range 1·6–7·5 mV), occasionally triggering one or two action potentials. During postinspiration, the membrane potentials of E-2 neurones were again slightly hyperpolarized and then gradually depolarized. During this phase, there was an absence of action potential discharge. Subsequent depolarization during the second stage of expiration led to a burst of impulses that ended with the onset of the inspiratory phase;

(iv) postinspiratory neurones hyperpolarized during early inspiration (mean $\pm$ S.D., $4{\cdot}2 \pm 2{\cdot}1$ mV; $n = 6$; range 2·8–8·4 mV) and then slowly depolarized during the remainder of inspiration. They were further depolarized (mean $\pm$ S.D., $4{\cdot}4 \pm 3{\cdot}3$ mV; $n = 6$; range 2–11 mV) during postinspiration and discharged a burst of action potentials. This was followed by a slight hyperpolarization during the latter part of the expiratory period;

(v) prevention of lung inflation caused a slight increase in peak amplitude and lengthening (10–20%) of phrenic and recurrent laryngeal nerve inspiratory activity (Fig. 1*A*). Concomitant with this increase in nerve activities, E-2 neurones showed an enhanced membrane hyperpolarization during inspiration and a pronounced slowing of depolarization during postinspiration (Fig. 1*A*); PI neurones exhibited a decreased firing rate during postinspiration (Fig. 1*B*).

Effects of lung inflation on respiratory rhythm and neuronal membrane potential following MK-801 administration

There was a tendency for the respiratory rate to increase slightly after intravenous application of MK-801 (Fig. 2). However, provided that the lungs were inflated during the neural inspiratory phase, the basic temporal pattern of phrenic nerve, recurrent laryngeal nerve as well as E-2 and PI membrane potential trajectories were not significantly altered by MK-801 (in doses sufficient to produce apneusis in the absence of lung inflation). The latter observation with respect to membrane potential

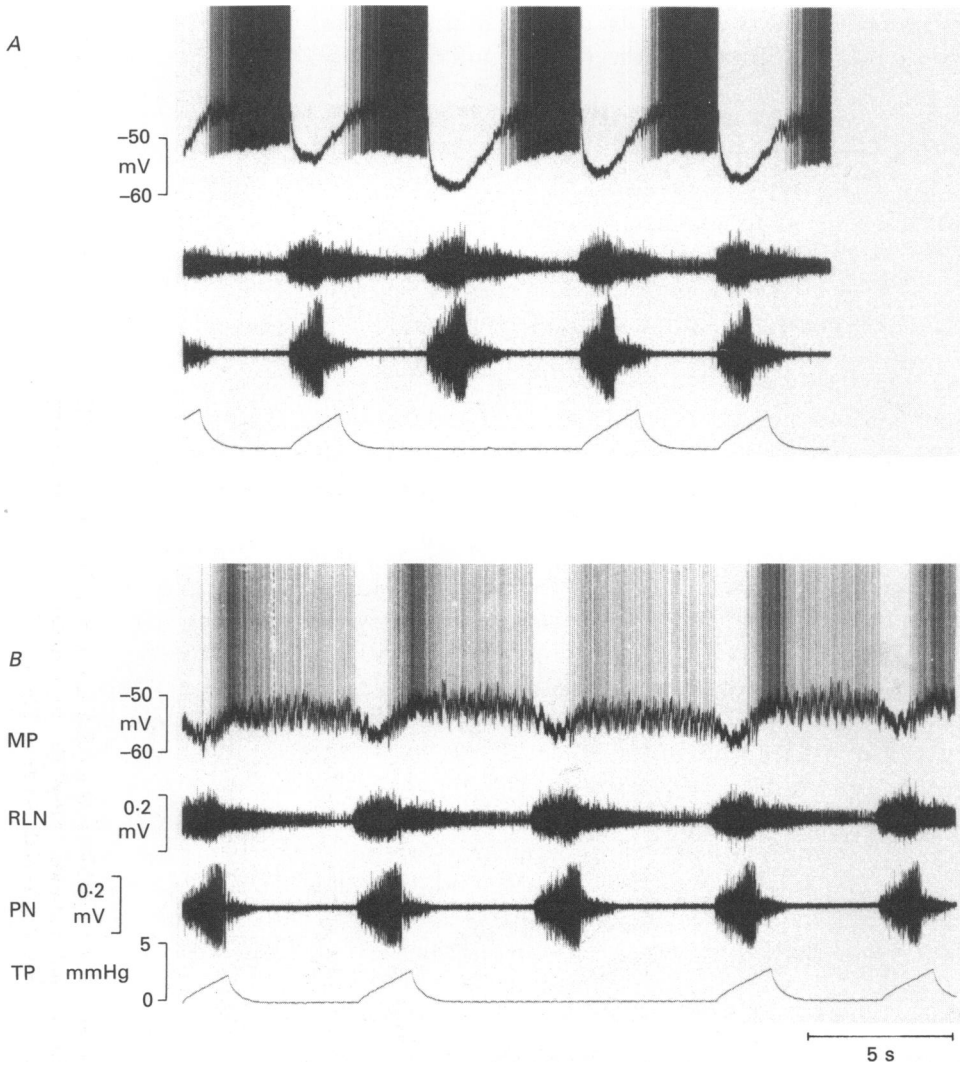

Fig. 1. Effect of non-inflation on membrane potentials of a late expiratory (E-2; *A*) and a postinspiratory (PI; *B*) medullary neurone. Phrenic nerve (PN) activity was used to trigger lung inflation (as measured by tracheal pressure: TP). During cycles when the 'cycle-triggered' inflation was withheld, PN and recurrent laryngeal nerve (RLN) activities increased and lengthened (10–20%). Inspiratory inhibition of the E-2 neurone (*A*) increased and lengthened. The PI neurone (*B*) was activated less and discharged at lower frequencies. MP = membrane potential.

trajectories is based on three cases in which cells were recorded continuously before and after MK-801 (e.g. Fig. 2), and comparison of cells recorded either before (e.g. Figs 1 and 5*A*) or after (Figs 3–8) MK-801.

Membrane potential trajectories during apneusis

During both control (pre-MK-801) and apneustic states (Fig. 3*A–D*), E-2 or PI neurones hyperpolarized at the beginning of inspiration. At high enough doses of MK-801 to cause apneusis during non-inflation (> 0·1 mg/kg; Fig. 3), the membrane

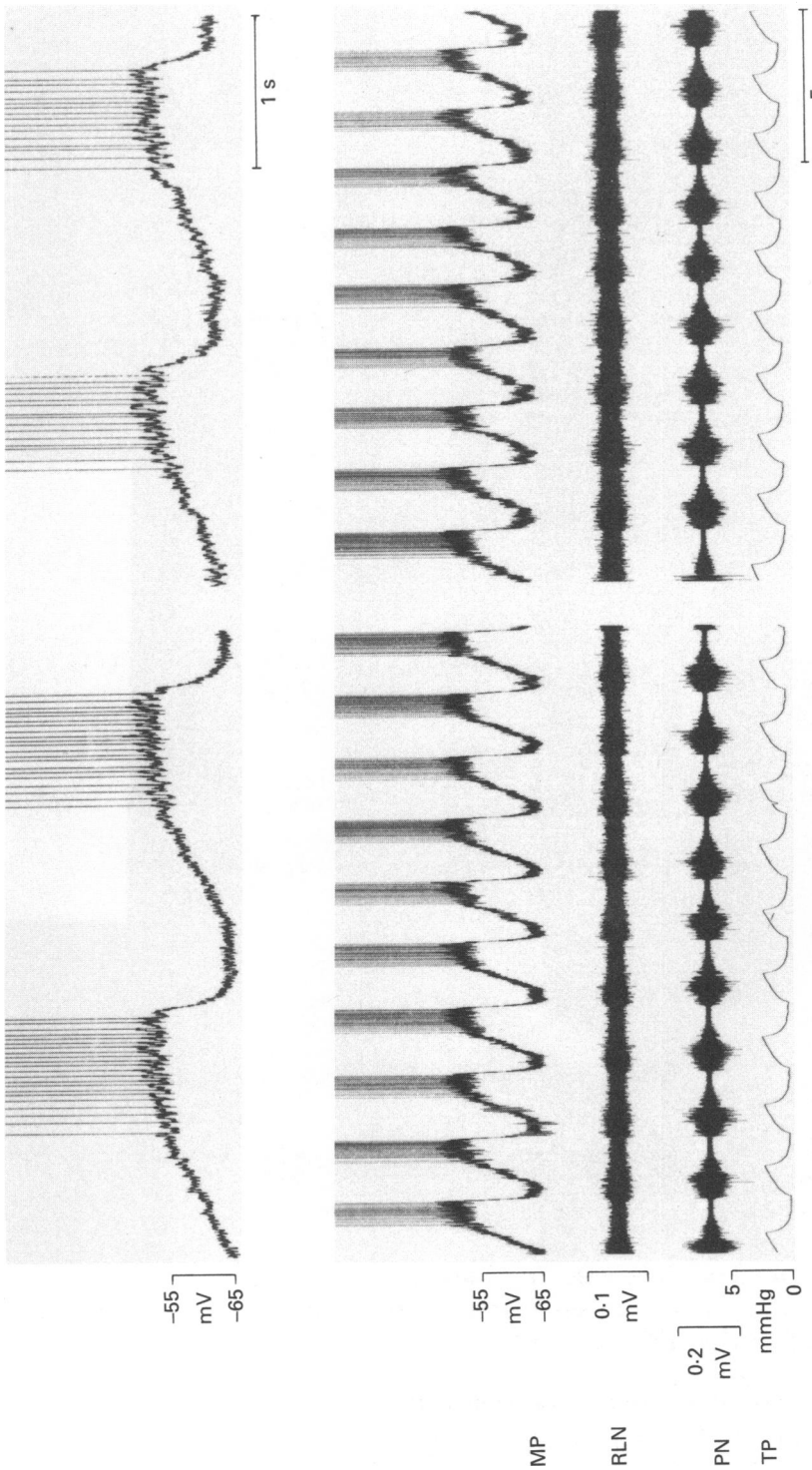

Fig. 2. Respiratory rhythm and membrane potential trajectories of an E-2 neurone before and after MK-801 application. The left column displays at higher (upper trace) and lower time resolution (lower traces) the unperturbed (self-generated) rhythm *ca* 1 min before, and the right column 4 min after application of 0·3 mg/kg MK-801. Abbreviations as in Fig. 1.

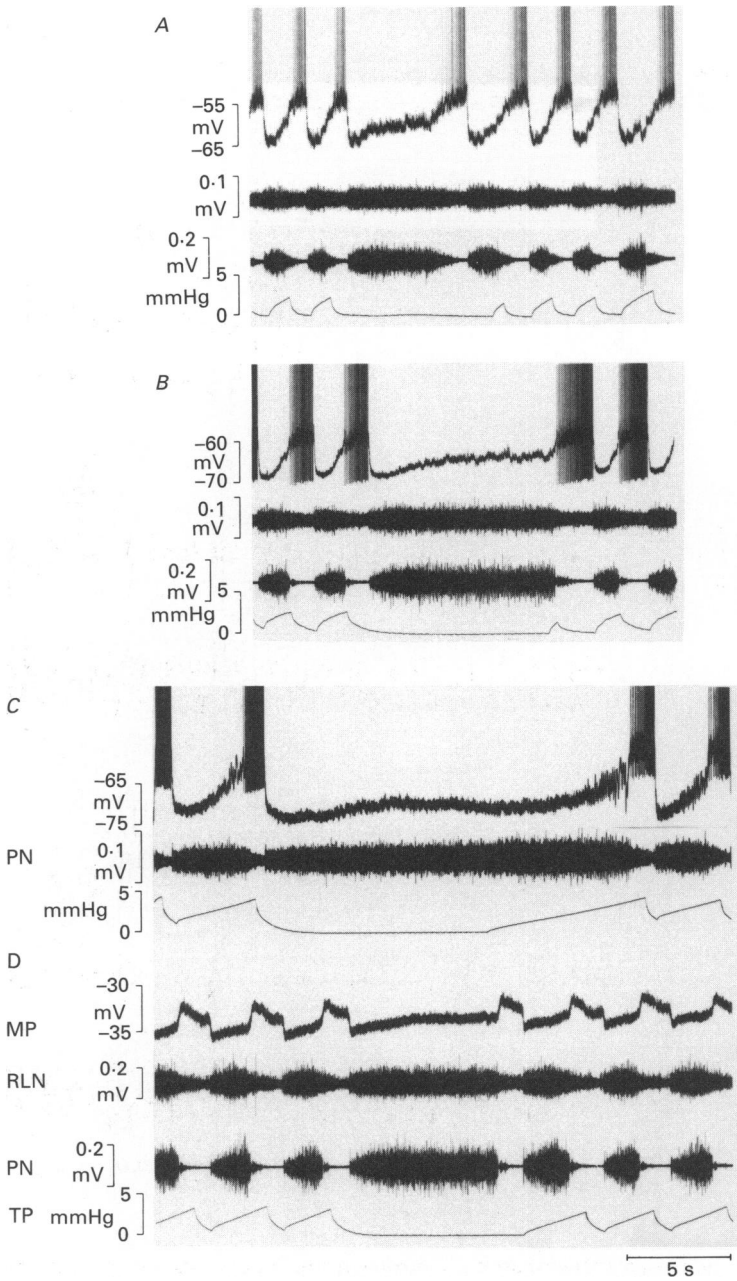

Fig. 3. Membrane potentials of three E-2 neurones (*A–C*) and a PI neurone (*D*) during apneusis induced by MK-801. *A* shows an example of self-terminated apneusis which started during the non-inflation test. *B* displays the common behaviour. *C* illustrates an occasional finding during long-lasting apneusis: during re-inflation, phrenic nerve activity increased and the membrane potential of the E-2 neurone reveals oscillations (usually at low frequency between 10 and 20 Hz) before the cell starts to discharge action potentials. *D* shows the membrane potential of a PI neurone whose spike discharge was inactivated. Abbreviations as in Fig. 1.

potentials of both E-2 (Fig. 3*A–C*) and PI (Fig. 3*D*) neurones remained hyperpolarized during the apneustic inspiratory period, independent of the duration. This was typically followed by a prolonged period of slow membrane depolarization which occurred at a considerably reduced slope (Fig. 3*A*, *B* and *D*). Occasionally,

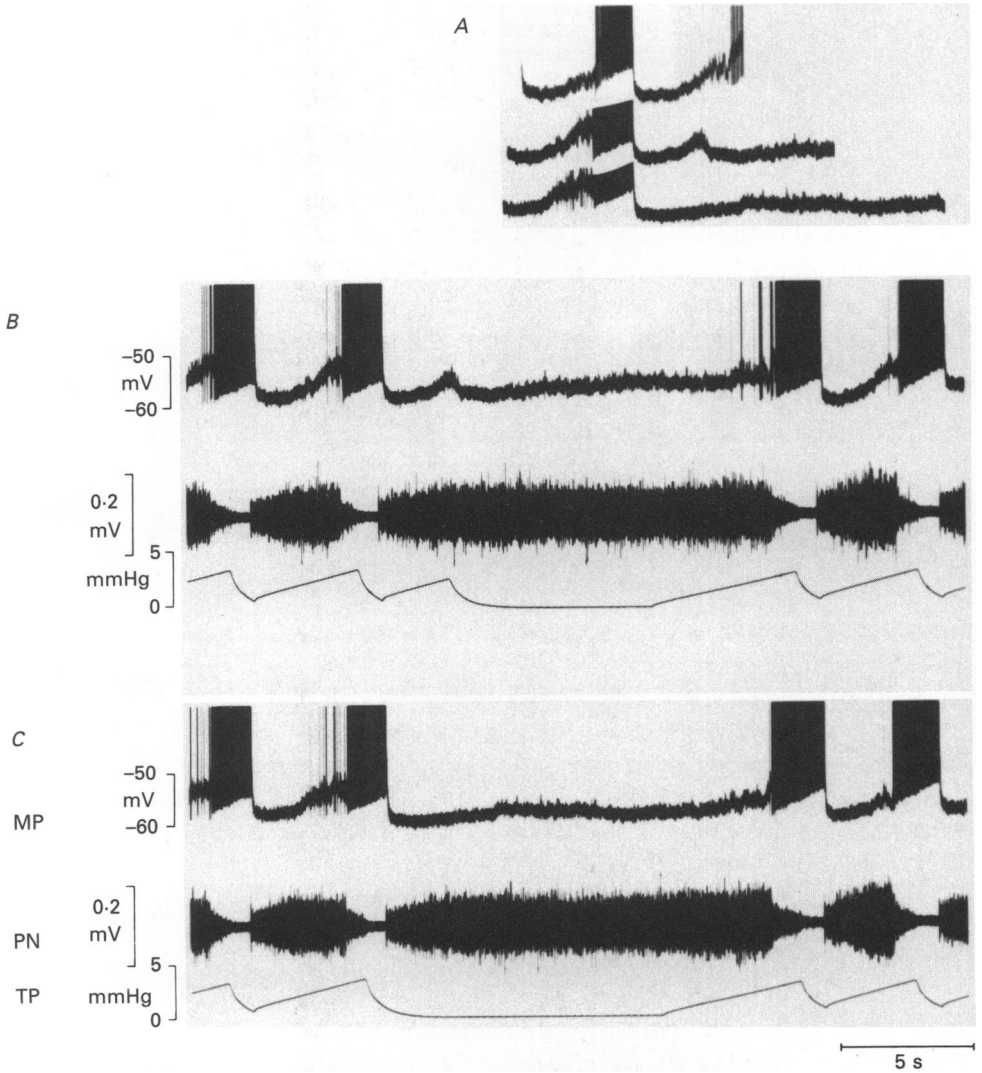

Fig. 4. Membrane potential of an E-2 neurone in which apneusis was initiated at different times of the respiratory cycle. Lung inflation was switched off late in inspiration in *B*, and after inspiration in *C*. *A* shows a comparison of the membrane potential trajectories during inspiratory inhibition that occurs during a normal cycle (upper trace) and apneustic periods of variable duration (lower two traces). Abbreviations as in Fig. 1.

particularly during long-lasting apneustic periods, there were small rehyperpolarizations correlated with slight increases in phrenic nerve activity (Figs 3*C* and 4*C*).

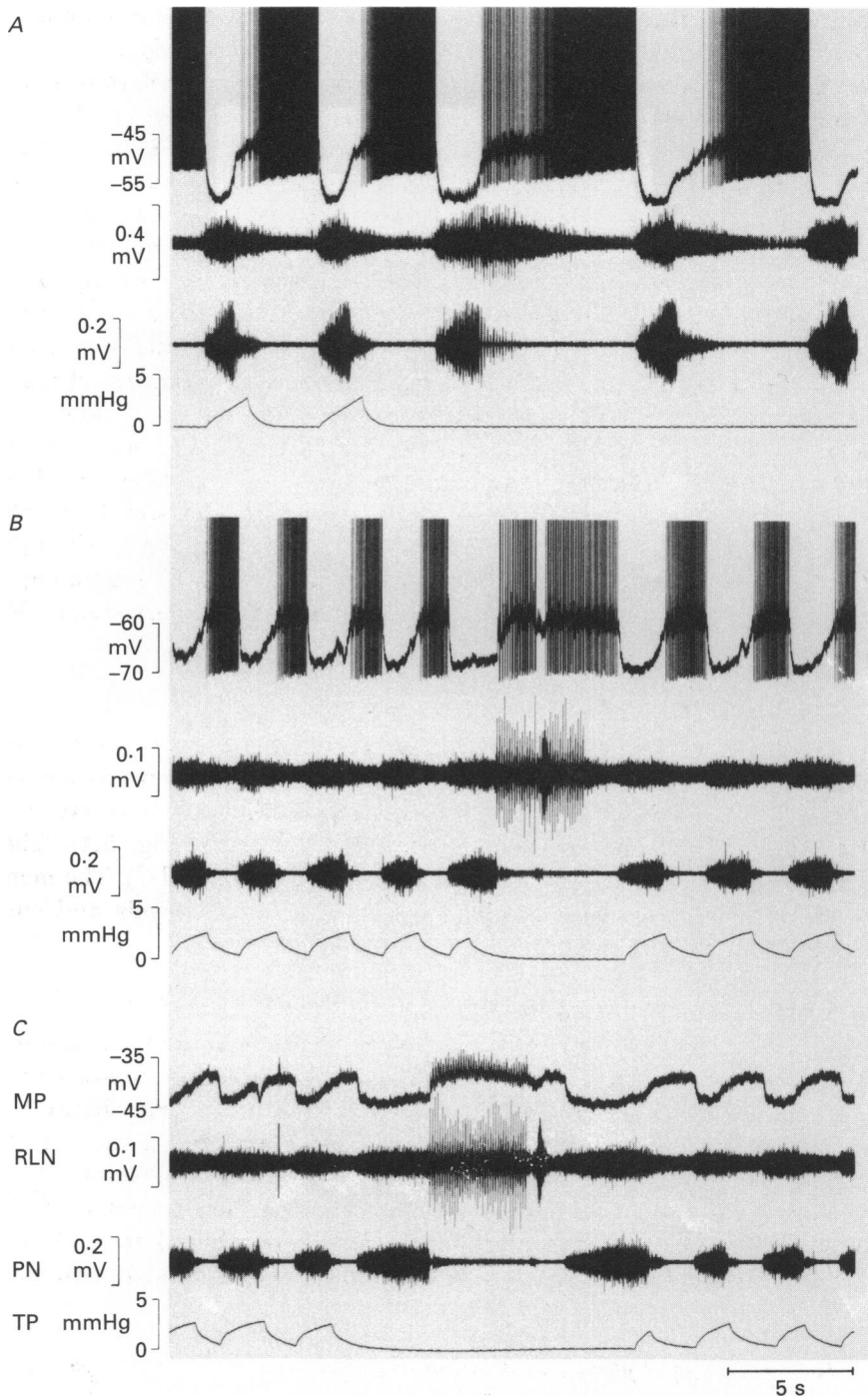

Fig. 5. Effects of electrical stimulation of the ipsilateral superior laryngeal nerve (SLN) on the membrane potentials of E-2 neurones before and during apneusis. *A* shows a control recording before MK-801 application. Note the stimulus artifacts. *B* and *C* show the effects of SLN stimulation in two other E-2 neurones during MK-801-induced apneusis. (Stimulus parameters: width 50 μs; in *A*, 1 V at 8 Hz; in *B*, 0·5 V at 10 Hz; in *C*, 0·5 V at 10 Hz). Abbreviations as in Fig. 1.

Membrane depolarization that followed the end of an apneustic inspiration was similar in cases when inspiration terminated either spontaneously (Fig. 3*A*) or by delayed lung inflation (e.g. Fig. 3*B*). The effects of pulmonary afferent activity on the membrane potential of E-2 neurones was related to the production of inspiratory termination (Fig. 4*A*). With lung inflation sufficiently long and intense to produce inspiratory termination (Fig. 4*B*, 2nd and 4th phrenic nerve burst; Fig. 4*A*, top trace), the E-2 neurones polarization pattern consisted of an initial abrupt hyperpolarization at the start of inspiration, a slow depolarization during late inspiration, a plateau potential during the postinspiratory phase followed by a burst of action potentials during the latter part of expiration. When a shorter duration inflation was applied (Fig. 4*B*, 3rd phrenic nerve burst), an apneustic inspiratory pattern developed. During the period of lung inflation, the trajectory of membrane potential was virtually identical to that of the previous normal (non-apneustic) phase (Fig. 4*A*, middle trace). However, following withdrawal of lung inflation and the resultant continuation of the phrenic burst, the membrane quickly hyperpolarized to the previous maximal level. Afterwards it repolarized gradually by approximately 2 mV, and then remained stable for the remainder of the apneustic inspiratory phase. This latter behaviour was comparable to that during an apneustic phase that resulted from no lung inflation (Fig. 4*C*, 2nd phrenic nerve burst; Fig. 4*A*, bottom trace).

Effects of laryngeal afferents before and after MK-801

Similar to lung stretch receptor afferents, laryngeal afferents have a powerful effect on respiration. Electrical stimulation of the superior laryngeal nerve at low voltages (range 0·5–1 V) and at low repetition rates (range 5–50 Hz) terminate a normal inspiration and prolong the subsequent expiratory interval (Fig. 5*A*). The same effect was exerted during MK-801-induced apneusis (Fig. 5*B* and *C*). The membrane potential of E-2 neurones was usually reset to depolarized levels and sustained synaptic inhibition was curtailed.

Neuronal input resistance during rhythmic ventilation and apneusis (Fig. 6)

An indication of the mechanism producing membrane hyperpolarization in E-2 and PI neurones is provided by measurements of neurone input resistances. Inhibitory postsynaptic potentials (IPSPs) which open Cl^- or K^+ channels, would reduce the neurone input resistance, whereas disfacilitation (reduced excitatory postsynaptic potentials) would increase this resistance. In E-2 neurones ($n = 11$), the input resistance was lowest during early inspiration and then increased until late in the expiratory period. From this high level, it subsequently fell abruptly to early-inspiratory levels and only slowly recovered to late-inspiratory levels throughout the apneustic phase.

Reversed IPSPs during rhythmic ventilation and apneusis

In order to determine whether the inspiration-related hyperpolarizations were due to Cl^--dependent IPSPs, Cl^- was injected into fourteen E-2 neurones to see if these hyperpolarizations could be reversed. Figure 7 shows an E-2 neurone prior to (Fig. 7*A*) and 15 s (Fig. 7*B*) after starting the injection of Cl^- ($-3{\cdot}6$ nA). After current injection, the hyperpolarizations during inspiration were converted to depolarizing

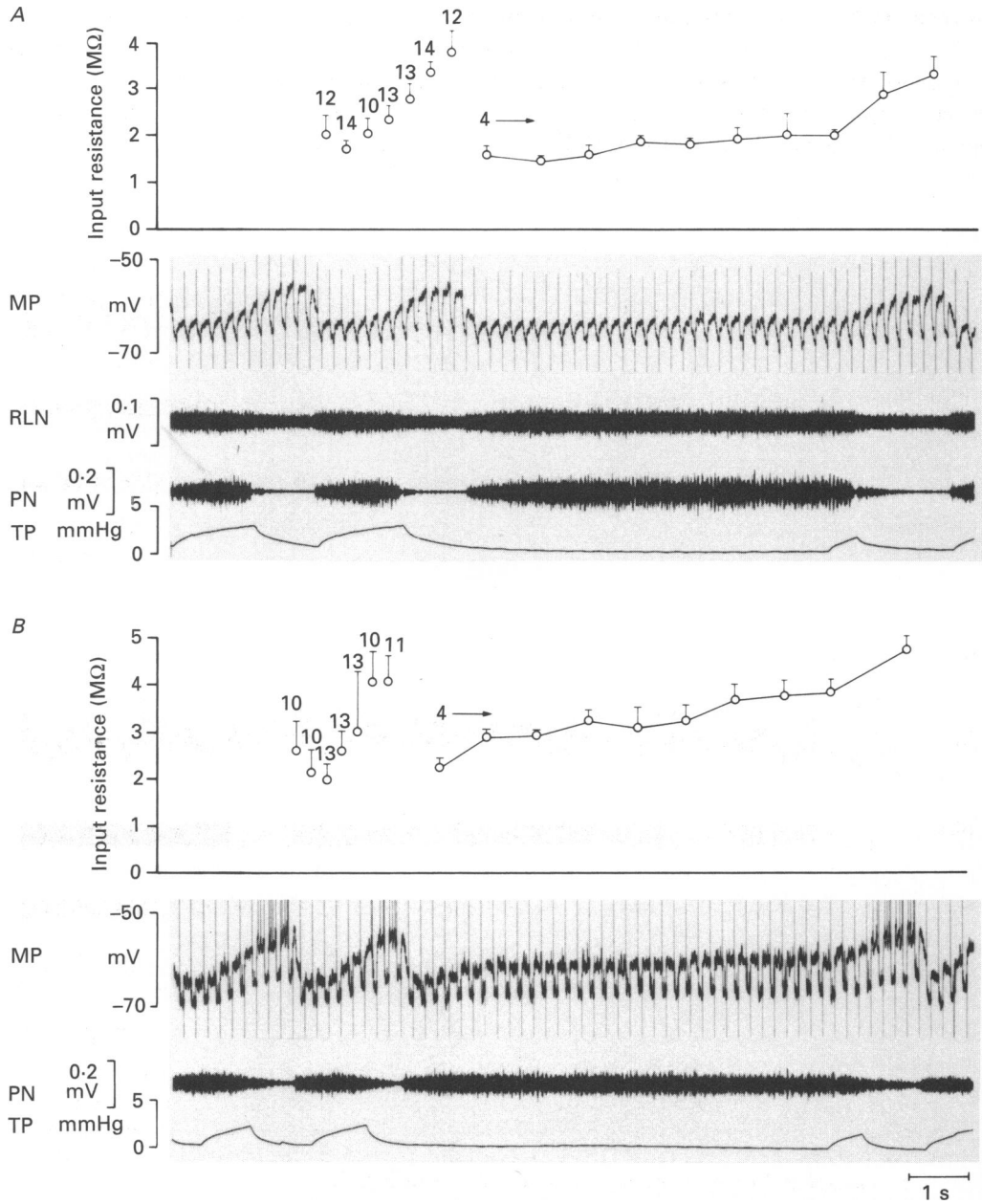

Fig. 6. Input resistance of an E-2 neurone during apneusis. Negative current pulses of 70 ms duration were injected at a repetition rate of 5 Hz (pulse intensity in *A* −2·7 nA; in *B* −1·7 nA). The graphs plot input resistance *vs.* time, and are synchronized with the lower traces showing the original measurements. Input resistance is given as mean ±S.D. For the control periods with lung inflation, these values were calculated by dividing several inspiratory cycles (in *A*: six cycles; in *B*: ten cycles) of about equal duration into seven equal time segments and determining the mean input resistance for a defined period within the cycle. For the non-inflation test, means ± S.D. values were calculated for four successive current pulses. The numbers above the mean ± S.D. values give the numbers of pulses used to compute each data point. Abbreviations as in Fig. 1.

waves, with an associated increase in synaptic noise. Another example of an E-2 cell following Cl^- injection (−2 nA) is shown in Fig. 7*C*, where reversal of the hyperpolarizing waves during rhythmic bursts of phrenic nerve activity, as well as during apneusis, are evident. During apneusis, the pattern of reversed IPSPs revealed a persistent inhibition of E-2 neurons (Fig. 7*C*).

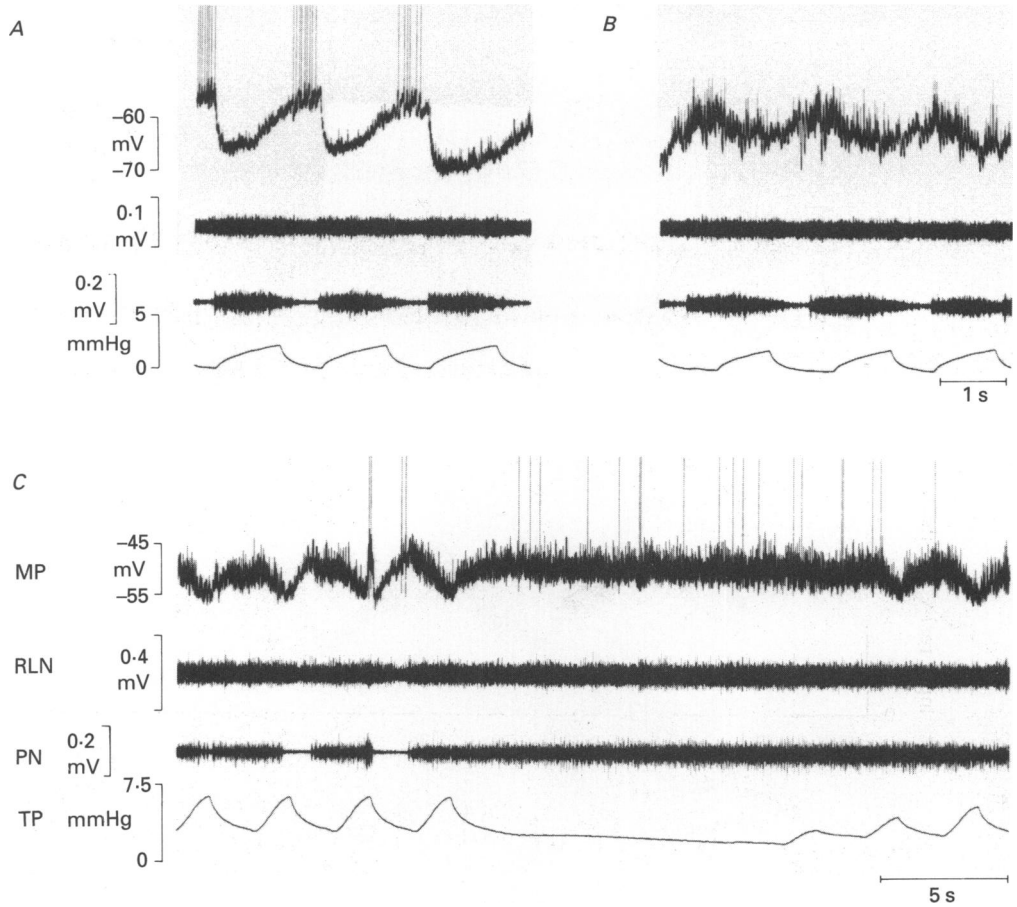

Fig. 7. Reversed inhibitory postsynaptic potentials in an E-2 neurone after MK-801 application. In *A*, *B* and *C*, the membrane potential is shown before, 15 s and 1 min (during apneusis) after start of negative current injection (−3·8 nA), respectively. The non-inflation test shown in *C* reveals a sustained pattern of reversed (Cl^- dependent) IPSPs arriving during apneusis. Abbreviations as in Fig. 1.

Oscillations in nerve activities and membrane potential during apneusis (Fig. 8)

During MK-801-induced apneusis, phrenic and recurrent nerve activities as well as E-2 membrane potentials often showed prominent oscillations at frequencies ranging between 10 and 20 Hz. Phrenic and recurrent laryngeal nerve activities exhibited large and regular oscillations which are best seen in the integrated traces (2nd and 4th from top). Such oscillations are also recognizable in the membrane potential of E-2 neurones. Averages of these three signals, triggered by oscillation peaks in low-pass filtered phrenic nerve activity (Fig. 8*B*), reveal that the oscillations were

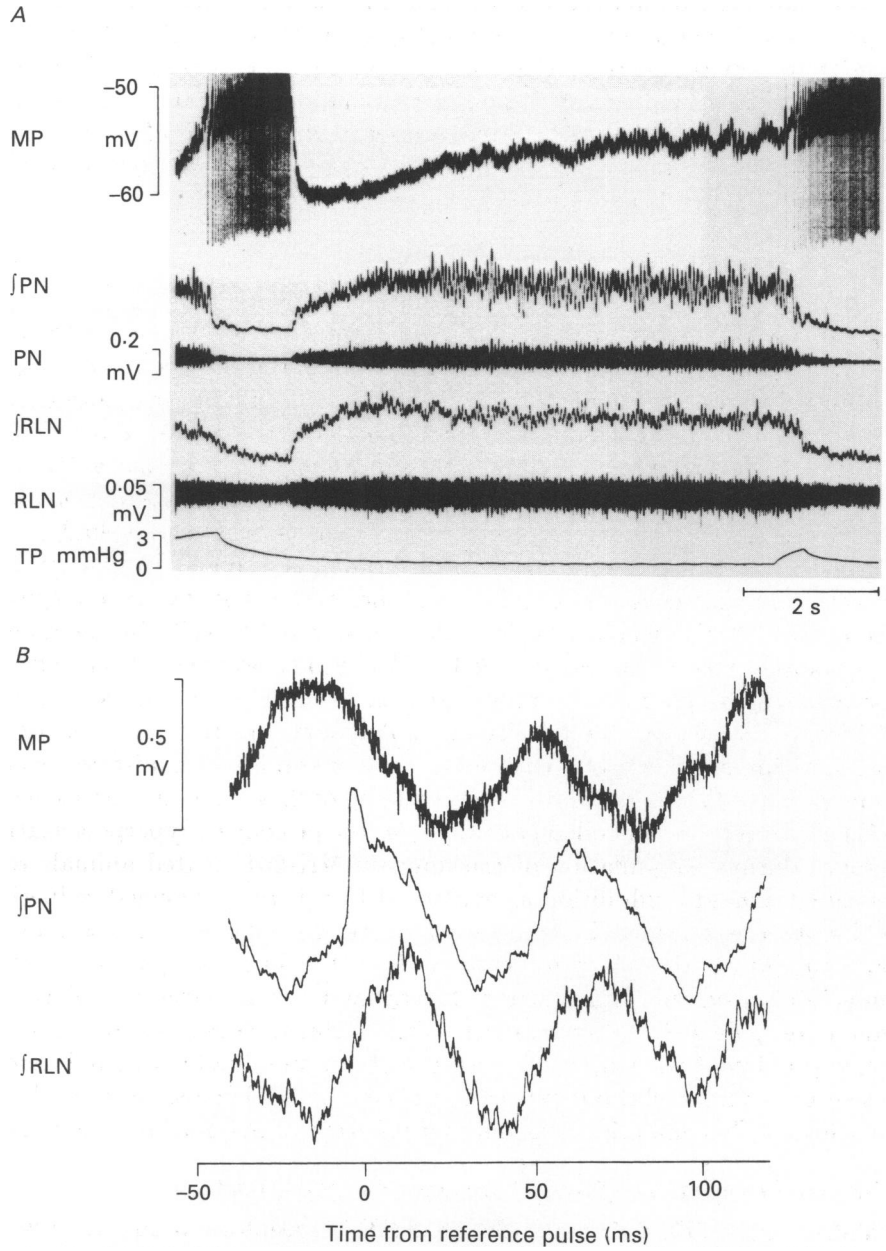

Fig. 8. Low-frequency oscillations that occur during apneusis. *A* illustrates a non-inflation test after MK-801 application leading to apneustic phrenic (PN) and recurrent laryngeal nerve (RLN) discharges oscillating at their plateau levels. The membrane potential (MP) of an E-2 neurone reveals similar oscillations. *B* shows an average of ten sweeps with membrane potential oscillations measured during the period of plateau PN activity where oscillations are prominent. Large-amplitude excursions of a low-pass filtered version (cut-off 200 Hz) of the PN signal were used as a trigger. These triggers were used as reference pulses at relative time zero on the lower time scale. For averaging PN and RLN activities as well as the MP of the expiratory neurone, signals were low-pass filtered with the same time constant of 15 ms. The averages reveal that the same rhythm appears in all three activities. Integrated PN and RLN activities are well in phase whereas a phase shift is

strongly correlated (for details see legend). The phrenic and recurrent nerve activities were in phase with each other; they were both out of phase, albeit not precisely at 180 deg, with the membrane potential of the E-2 neurone. The period of the averaged correlated oscillations was about 60 ms, corresponding to their mean frequency. Occasionally, oscillations were particularly pronounced at the end of a longer-lasting apneustic activity (Fig. 3*C*) or when apneusis was forcibly terminated by large lung inflations or subthreshold superior laryngeal nerve stimulation.

DISCUSSION

We examined and compared the membrane potentials of medullary respiratory neurones during normal and apneustic breaths in anaesthetized cats. We made the following principal observations: (1) the characteristic membrane potential trajectories of E-2 and PI neurones were not affected by MK-801 during normal respiratory cycles in which lungs were inflated. Under these conditions, activation of NMDA receptors does not seem to be essential for the synaptic interactions within the spinomedullary respiratory network; (2) NMDA-receptor blockade, which evokes apneusis when the lungs are not inflated, produced consistent changes in the synaptic activation patterns of E-2 and PI neurones of the caudal ventral respiratory group. In particular, the depolarization pattern of E-2 and PI cells during apneustic inspiratory phases, was consistently retarded. We argue below that this is indicative of the process responsible for the prolongation of the inspiratory phase; (3) during MK-801-induced apneusis, the ability of pulmonary stretch receptor or low-threshold superior laryngeal afferent activation to terminate inspiration was not compromised. We conclude that synaptic processing of these afferent signals does not involve NMDA-receptor-controlled pathways; (4) the prolonged hyperpolarization in E-2 neurones during non-inflation of the lungs in MK-801-treated animals results from persistent synaptic inhibition as evidenced by: (i) the prolonged reduction of neurone input resistance, and (ii) reversal of IPSPs following intracellular Cl^- injection; (5) during the plateau phase and at the end of apneusis 10–20 Hz oscillations in both phrenic and recurrent laryngeal nerve activities as well as in the membrane potentials of E-2 neurones were often present. These oscillations became particularly prominent when apneusis was forcibly terminated by lung inflation. We hypothesize that the oscillations reflect the effects of NMDA-receptor blockade in central pathways that underlie reversible termination of inspiration (see below).

Mechanisms and localization of NMDA-receptor-controlled functions

The finding that MK-801 blockade of NMDA receptors produces apneustic breathing patterns demands an explanation of three essential questions. (1) What changes occur in the inspiratory termination mechanisms to prolong inspiration? (2)

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apparent between the latter and the averaged MP of the E-2 neurone. The period of the oscillations is about 60 ms indicating an oscillation frequency of 16–17 Hz. This is confirmed by simply counting oscillatory cycles per time unit in the integrated PN activity, in which 16 to 17 cycles occurred throughout the apneustic (plateau) period. RLN and ∫RLN = recurrent laryngeal nerve activity in original and integrated form; PN and ∫PN = phrenic nerve activity in original and integrated form. Other abbreviations as in Fig. 1.

Why does the inspiratory activity stabilize at a plateau at which it tends to oscillate? (3) Which respiratory neurones are affected by NMDA receptors?

Inspiratory termination (*Fig. 9*). NMDA-receptor blockade causes prolongation of inspiratory activity because of disturbance of the processes leading to termination of

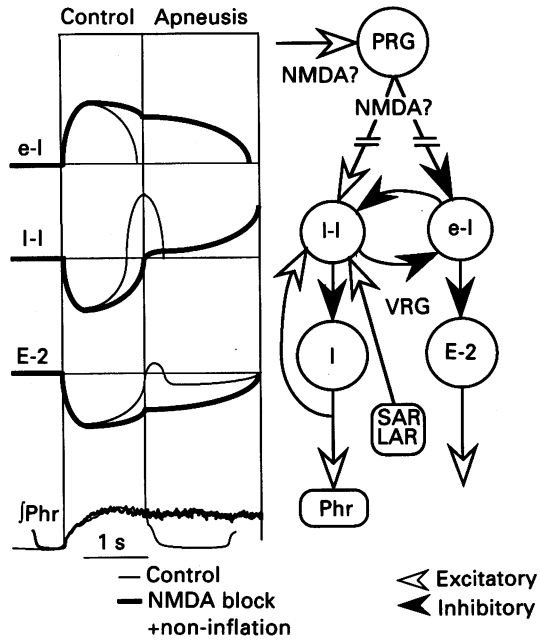

Fig. 9. Scheme summarizes and interprets the results. For description see text. PRG = pontine respiratory group; VRG = ventral group of respiratory neurones; E-2 = late-expiratory neurones; I = inspiratory neurones; e-I = early inspiratory neurones; l-I = late inspiratory neurones; Phr = phrenic motoneurones or activity; SAR = slowly adapting pulmonary receptors (stretch receptors) of the lung; LAR = laryngeal receptors.

inspiration. Two successively activated mechanisms are hypothesized to terminate inspiration in adult mammals. The first process, which normally defines late inspiration, reversibly inhibits the inspiratory (ramp) pattern-generating network (Bradley, von Euler, Marttila & Roos, 1975; Feldman, Cohen & Wolotsky, 1976; Younes *et al.* 1978; Remmers *et al.* 1979; Baker & Remmers, 1980; Richter, 1982; Ballantyne & Richter, 1984; Richter, Ballantyne & Remmers, 1986). Neurones with a late-inspiratory discharge are postulated to transmit this inhibition (Feldman *et al.* 1976; Cohen & Feldman, 1977; Ballantyne & Richter, 1984; Cohen, Feldman & Sommer, 1985). The late-inspiratory neurone discharge pattern is proposed to be synaptically controlled by excitatory inputs from augmenting inspiratory neurones and inhibitory inputs from early-inspiratory neurones (Richter, 1982). The interaction between early-inspiratory and late-inspiratory neurones, therefore, seems critical for the determination of inspiratory duration (Merrill, 1974; Richter *et al.* 1986). That is, reversible inhibition of inspiration would not normally begin until the inhibitory effects of early-inspiratory neurones declines, allowing late-inspiratory neurones to discharge. Late-inspiratory neurones also receive excitatory inputs from

slowly adapting pulmonary or laryngeal afferents, whose activation shortens inspiration (Iscoe *et al.* 1979; Richter *et al.* 1987). Subsequent to the onset of the reversible decline of inspiratory activity, an 'irreversible' termination of inspiration follows. The time course of this latter inhibition suggests that it results from a potent and widespread inhibitory action of PI neurones (Richter *et al.* 1986; Remmers, Richter, Ballantyne, Bainton & Klein, 1986; Richter *et al.* 1987; not illustrated in Fig. 9).

We presume that it is the reversible late-inspiratory termination mechanism that is affected by MK-801, leading to apneusis when the lungs are not inflated. We base this conclusion on the observation that during apneusis there is no change in either: (i) the relative timing of the onset of PI neuronal discharge, i.e. they still do not discharge until after the marked decline in phrenic nerve activity, or: (ii) the timing or initial step size of postinspiratory inhibition of E-2 neurones.

The observation that the lung inflation reflex remains potent during blockade of NMDA receptors suggests that late-inspiratory neurones and their excitatory peripheral inputs mediating inspiratory phase termination are not affected (Fig. 9). The presumed delayed discharge of late-inspiratory neurones during apneusis could be due to changes in early-inspiratory neuronal discharges (see below) or to changes in the synaptic drive of late-inspiratory neurones originating from other brain structures (see below).

Although we did not directly record from early inspiratory neurones, the inspiratory hyperpolarization pattern of E-2 neurones provides an indirect measure. This polarization pattern suggests synaptic inhibition by early-inspiratory neurones, which normally discharge at high frequency at the beginning of inspiration followed by a decline to low discharge rates during late inspiration (Ballantyne & Richter, 1986). During apneusis induced by withholding lung inflations, the rate of membrane repolarization of E-2 neurones was markedly reduced. This indicates slowing of the removal of inhibition (disinhibition) as evidenced by: (i) a steady decrease in membrane conductance as inspiration progressed (Fig. 6), and (ii) reversal of membrane hyperpolarization during inspiration after Cl^- injection (Fig. 7). It is also possible that this is due to a change in intrinsic membrane properties of E-2 neurones, but we propose this is unlikely since: (i) their inspiratory hyperpolarization pattern during lung inflation was similar before and after MK-801, and (ii) the peak inhibition of E-2 neurones during early inspiration was similar before and after MK-801 administration, regardless of whether or not the lungs were inflated. However, the peak membrane hyperpolarization of E-2 neurones at the beginning of apneusis was unchanged compared to control cycles, suggesting that the peak discharge of early-inspiratory neurones remained unchanged after MK-801 application. Prolongation of early-inspiratory inhibition of E-2 neurones during apneusis indicates that early-inspiratory neurone discharge declined more slowly. These changes in early-inspiratory neurones discharge should result in prolonged inhibition of late-inspiratory neurones, whose suppressed discharge would be unable to terminate inspiration at the normal time (Fig. 9).

The presumptive changes in early-inspiratory neuronal firing could involve: (i) alteration of intrinsic membrane properties that control discharge adaptation. For example, the burst pattern of these cells is affected by Ca^{2+}-dependent mechanisms

(Mifflin, Ballantyne, Backman & Richter, 1985; Champagnat, Jacquin & Richter, 1986, Richter *et al.* 1986; Mifflin & Richter, 1987) and could be modulated (or controlled) by NMDA receptors, which gate channels that pass Ca^{2+} (Lambert & Heinemann, 1986; MacDermott, Mayer, Westbrook, Smith & Barker, 1986; Ascher & Nowak, 1988); (ii) changes in synaptic inputs originating from other parts of the network, such as reduction of recurrent inhibition by late-inspiratory neurones (Richter, 1982; Lawson, Richter, Ballantyne & Lalley, 1989). The mechanism by which NMDA receptors could directly modulate this inhibition is not obvious.

PI neurones do not seem to be directly involved in the production of apneusis, since the postinspiratory discharge in phrenic nerves and postinspiratory inhibition of E-2 neurones was undisturbed following MK-801 administration. Graded reduction of early-inspiratory discharge can lead to disinhibition of PI neurones and explain their early onset of discharge extending into the apneustic period (see Fig. 3*A* and *C* in Foutz *et al.* 1988).

Inspiratory plateau activity and the origin of the oscillations during apneusis. Late-inspiratory neurones are tonically active in some types of apneusis (Remmers *et al.* 1979; Baker & Remmers, 1980, J. L. Feldman, U. Windhorst, K. Anders, M. Bellingham & D. W. Richter, unpublished observations). Plateauing of inspiratory activity superimposed by oscillations of inspiratory activities may be expected when excitatory inspiratory drive and late-inspiratory inhibition compete with each other, especially if the inhibition is attenuated (see below). Recycling interaction between the inspiratory drive and late-inspiratory inhibition (Fig. 9) could manifest itself in 10–20 Hz waxing and waning of inspiratory discharge during (Fig. 8) or at the end (Fig. 3*C*) of apneusis.

Neurones that seem to be controlled by NMDA receptors

Either blockade of NMDA receptors (Foutz *et al.* 1988) or lesions of the pontine respiratory group in anaesthetized cats (Feldman & Gautier, 1976) produce apneusis in the absence of pulmonary stretch receptor activity associated with lung inflation. Neither of these perturbations affects the ability of pulmonary stretch receptors or laryngeal afferents to produce inspiratory termination, nor is the ability to generate an initially normal inspiratory burst affected. The finding that MK-801 blockade of NMDA receptors produces apneustic breathing patterns when the lungs are not inflated points to the functional significance of pontine structures, which can exert an inhibitory influence upon the medullary respiratory neurones (Fournier, Richter & Feldman, 1987). Pontine respiratory neurones have limited phasic activity under normal conditions, as they are effectively blocked by vagal afferents (Feldman *et al.* 1976), and inspiratory phase termination seems to be controlled by medullary mechanisms that receive pulmonary and laryngeal afferent input (Feldman, 1986). These mechanisms do not seem to involve NMDA-receptor activation, since breathing is not apneustic when the lungs are inflated when NMDA receptors are blocked (Foutz *et al.* 1988). However, when vagal afferent signals are blocked, as in the case of non-inflation, pontine structures become active and contribute to termination of inspiration (Feldman *et al.* 1976). We conclude that the pontine respiratory group contribution to inspiratory termination: (i) requires NMDA-receptor activation (Foutz *et al.* 1988), and (ii) acts on the medullary respiratory

network by delays in the reduction of early-inspiratory neuronal discharge and/or in the activation of late-inspiratory neuronal discharge (Fig. 9).

We suggest two hypotheses: (1) NMDA-receptor blockade interferes with the pontine respiratory group contribution to inspiratory termination in the absence of pulmonary stretch receptor activity. We cannot speculate about the precise location of the NMDA receptors critical for inspiratory termination in the absence of lung inflation. They could be in circuits affecting pontine respiratory group activity, i.e. on pontine respiratory group neurones themselves, or on neurones which receive pontine respiratory group input, ultimately projecting to early- and late-inspiratory neurones to affect their discharge pattern. Since the specific actions of the pontine respiratory group seem to be different in respiratory cycles with and without lung inflation (Feldman *et al.* 1976), these NMDA-receptor controlled pathways may not be active during cycles with normal lung inflation (Feldman, Smith, Ellenberger, Connelly, Liu, Greer, Lindsay & Otto, 1990*a*); (2) there are at least two separate pathways capable of producing inspiratory termination (Feldman, Smith, McCrimmon, Ellenberger & Speck, 1988): (i) a central pathway affected by NMDA-receptor blockade or pontine respiratory group lesions, and (ii) one or more afferent pathways activated by signals from pulmonary stretch receptors and laryngeal afferents.

In conclusion, neither the generation of the characteristic membrane potential patterns during the respiratory cycle, nor the production of motor output was affected by MK-801. Therefore, NMDA-receptor activation is not essential for synaptic interactions within the spinomedullary network involved in generating the respiratory pattern or in producing motoneuronal output. The synaptic efficacy within the reflex circuit from laryngeal afferents to E-2 neurones and phrenic nerve also is not compromised by NDMA-receptor blockade. NMDA receptors are critical in central circuits required to terminate inspiration in the absence of peripheral input. These circuits are likely to include a pontine respiratory group-mediated control of early- and late-inspiratory neurones, possibly in the pre-Bötzinger Complex (Feldman & Smith, 1989; Smith, Greer, Liu & Feldman, 1990; Smith, Ellenberger, Ballanyi, Richter & Feldman, 1991; Schwarzacher, Smith & Richter, 1991).

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