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THE NON-UNIFORM CHARACTER OF EXPIRATORY SYNAPTIC ACTIVITY IN EXPIRATORY BULBOSPINAL NEURONES OF THE CAT

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SUMMARY

1. Intracellular recordings were made from caudal medullary expiratory neurones in pentobarbitone-anaesthetized, vagotomized and artificially ventilated cats. The sample consisted of thirty-three bulbospinal neurones and seven neurones which were not antidromically excited from either the spinal cord (C2–C3) or vagus nerve.

2. Their rhythmic activity consisted of an alternating inspiratory hyperpolarization due to Cl^--dependent inhibitory post-synaptic potentials (i.p.s.p.s) (Mitchell & Herbert, 1974) and an expiratory depolarization. The precise shape of the expiratory depolarizing wave varied within a given neurone depending on the over-all pattern of respiration. This variation extended from a smoothly developing depolarization, continuous throughout its course, through an intermediate state in which depolarization proceeded in two stages with a definite transition between them, to a final state in which the early part of expiration was occupied by a distinct hyperpolarizing component to the membrane potential trajectory.

3. Under conditions of a brisk phrenic nerve discharge, these variations in the shape of the membrane potential profile were related to the time course and intensity of post-inspiratory discharge in the nerve. However, other factors (depth of anaesthesia and stimulation of laryngeal receptors) could influence the time course of the membrane potential profile of expiratory neurones independently of post-inspiratory phrenic discharge.

4. In five of fifteen neurones which were tested, early expiration was occupied by a rapidly developing, decrementing wave of Cl^--dependent i.p.s.p.s (post-inspiratory i.p.s.p.s). These i.p.s.p.s were present only under conditions of a strong phrenic rhythm (large amplitude, fairly rapid phrenic discharge). They became weaker and ultimately disappeared when the level of anaesthesia was deepened and the phrenic rhythm became slower. Under these conditions, the post-inspiratory wave of i.p.s.p.s could be restored by stimulation of the superior laryngeal nerve.

5. Adequate stimulation of presumed 'irritant' laryngeal receptors elicited post-inspiratory i.p.s.p.s in seven of ten neurones tested which initially showed either no post-inspiratory i.p.s.p.s or possibly just a weak pattern.

6. In ten of fifteen neurones tested, the responses to current injection revealed clear differences in membrane potential behaviour in early and late expiration, which became intensified following stimulation of the superior laryngeal nerve. There was

no clear evidence of post-inspiratory i.p.s.p.s in these neurones and it is possible that the synaptic feature dominating the membrane potential profile in early expiration in these neurones is a low level of excitatory input.

7. We conclude that expiratory synaptic activity is not uniform throughout its course but that its earlier (post-inspiratory) and later stages are differentiated to a greater or lesser extent. We suggest that the prominence of this differentiation is related to the over-all pattern of respiration as well as, for instance, the influence of activity in laryngeal afferents, and that the modifiable character of early expiratory synaptic activity serves primarily to modulate the structure of the expiratory burst.

INTRODUCTION

In two recent studies of the synaptic control of medullary inspiratory neurones, we have given evidence that the respiratory rhythm is commonly represented in these neurones by a sequence which is composed of three phases of synaptic activity rather than simply the two that might have been expected on the basis of a bi-stable oscillation between inspiratory and expiratory neural activity (Richter, 1982; Ballantyne & Richter, 1984). This sequence results from the subdivision of the expiratory interval in these neurones into two phases or stages, distinguished not only by the synaptic activity but typically also by the relationship of each stage to particular features of the respiratory motor output recorded in the phrenic nerve. The distinctive character of each stage is well illustrated by the behaviour of inspiratory bulbospinal neurones located in either of the two main (dorsal and ventral) groups of medullary respiratory neurones. At moderate frequencies of respiration the early stage of expiration is occupied by a more or less gradual decline in the intensity of synaptic excitation from the peak which is reached towards the end of inspiration. The time course of this decline follows approximately that of the post-inspiratory component of phrenic nerve activity, at least when this is well developed. The later stage of expiration is occupied by an incrementing pattern of synaptic inhibition (i.p.s.p.s), which thus usually coincides with the 'silent' interval in the nerve, and which ceases abruptly with the onset of the next phase of inspiration (see also, Richter, Camerer, Meesmann & Röhrig, 1979).

A synaptic rhythm of this kind is open to several kinds of interpretation. Inasmuch as the two-stage expiratory interval in bulbospinal neurones manifests itself as a *delayed* onset to what is essentially a ramp-like pattern of expiratory inhibition, one possibility is that the early stage of expiration constitutes a distinct 'phase' of the respiratory cycle in the sense that it serves to introduce a variable delay between the point at which the inspiratory 'ramp generator' is switched off, and the point at which an expiratory 'ramp generator' is switched on (Richter & Ballantyne, 1983). More recently, it has become apparent that not only the magnitude of this delay to expiratory inhibition is variable, but the character of the associated synaptic activity may be extensively modified in response to stimulation of various kinds of centrally and peripherally originating afferents (D. W. Richter, D. Ballantyne & J. E. Remmers, unpublished data). This naturally extends considerably the range of potential significance of events occurring in the early part of expiration, and reveals an important limitation of the hypothesis mentioned above, namely that the signi-

ficance of events immediately following the transition from inspiration to expiration is likely to be very different in different classes of respiratory neurone. At this stage, it would seem desirable to establish a catalogue of neurones which could be searched for some kind of consistency in the way their expiratory synaptic activity is organized in different conditions. A start has been made in this direction by the preliminary survey of neurones made by Richter (1982) and the present study represents an extension of this survey to include a detailed account of the expiratory synaptic activity of expiratory bulbospinal neurones located in the nucleus retroambigualis.

The main aim of this paper was an examination of the expiratory phase membrane potential trajectory in these neurones, following their release from inspiratory inhibition (Mitchell & Herbert, 1974; Hildebrandt, 1974), for evidence of a division comparable in terms of timing to that present in inspiratory neurones. Such a division was found in most neurones, and the second part of this paper is concerned with the nature of this division. Some of the present results have been published previously in abstract form (Ballantyne & Richter, 1982*b*).

METHODS

The experiments were performed on forty cats weighing 2·2–4·0 kg. They were anaesthetized with sodium pentobarbitone (35–40 mg/kg initial dose I.P., plus supplementary doses I.V. as required), paralysed with gallamine triethiodide and artificially ventilated via a tracheal cannula on O_2-enriched air to a minute volume maintaining end-tidal CO_2 in the range 3–5 vol. %. The tracheal cannula was fitted with a separate port for the introduction of water or weakly alkaline solution into the larynx. Respiratory movements of the brain stem were prevented by bilateral pneumothorax, and a positive end-expiratory pressure of *ca.* 2 cmH_2O was established by inserting a resistance into the expiratory outlet from the pump. Blood pressure was recorded via a femoral artery catheter and was monitored throughout the experiment. Rectal temperature was usually well maintained at 37–38 °C by external heating.

Stimulation and recording

The phrenic nerves were severed bilaterally in the neck and their proximal stumps prepared for recording. All of the recordings in this series of experiments were obtained after the vagus nerves were severed bilaterally high in the neck. The proximal stump of the vagus ipsilateral to the medullary recording site was mounted on bipolar stimulating electrodes, as was the intact main trunk of the ipsilateral superior laryngeal nerve. For the identification of expiratory bulbospinal neurones, laminectomy was performed to expose C2–C3, at which level their descending axons form a fairly discrete tract (Merrill, 1970, 1971), and four bipolar stainless-steel electrodes with tip diameters of 100 μm were inserted into the ventrolateral region of the cord. The medulla was exposed by occipital craniotomy, the membranes removed and a 'patch' prepared on the surface for inserting the micro-electrode. The area under the 'patch' was stabilized by applying a small pressure ring (diameter 1 mm) to the surface. It was usually unnecessary to retract the cerebellum.

For experiments involving the intracellular injection of Cl^-, micro-electrodes were filled with a 3 M-KCl solution and their tips carefully broken back to achieve a final d.c. resistance of 10–20 MΩ in Ringer solution. In other experiments (Figs. 2, 3, and 10*A* and *B*), they were filled with a 2 M-K citrate solution and had d.c. resistances of 20–50 MΩ. Intracellularly recorded potentials were amplified with compensation for stray capacitance, and with a bridge circuit for current injection across the recording micro-electrode. The balanced state of the bridge was monitored by applying rectangular current pulses to the micro-electrode. A d.c. record of membrane potential was displayed continuously at low and variable high gain on a pen-writer on which there was also a trace for phrenic nerve activity, usually displayed in 'integrated' form (time constant 0·2–0·3 s). All data were stored on magnetic tape (d.c. to 5 kHz frequency response at the speed used) and the records presented here include tape replays onto the pen-writer and *X*–*Y* plots of the output from a digital oscilloscope (Figs. 3*A–C* and 8*A–C*).

Identification of neurones and selection of data

Expiratory neurones were located 2·5–3·0 mm lateral to the mid line and were sampled from obex level to *ca.* 3 mm caudal to obex. Their depths below the dorsal surface ranged from 1·6 to 2·9 mm. Characteristically, they were encountered in small (two-to-three cell) clusters along a dorsoventral track, ventral and usually somewhat lateral to clusters of expiratory fibres. The location of these recording sites corresponds essentially to the 'retroambigual region' of the ventral group of respiratory neurones (Merrill, 1970; Bianchi, 1971; Kreuter, Richter, Camerer & Senekowitsch, 1977; Kalia, 1981) but in a few experiments conducted for other purposes we verified the general location of these neurones directly by the intracellular injection of Lucifer Yellow.

During exploratory tracking through the medulla, expiratory neurones were identified as such, typically prior to impalement, by their rhythmic discharge in phase with the expiratory interval in phrenic nerve activity. Expiratory neurones which were antidromically excited by vagus (recurrent laryngeal or superior laryngeal) nerve stimulation were excluded from the sample, but these were in any event not very common within these mediolateral co-ordinates or at levels more superficial than *ca.* 3 mm from the dorsal surface. Neurones regularly showing a decrementing pattern of expiratory discharge ('early expiratory' (Feldman & Cohen, 1978) or 'post-inspiratory' neurones (Richter, 1982)) were not included with the sample, but a further criterion was used to distinguish between these neurones and the 'late expiratory' neurones which were selected, namely their different response to stimulation of the superior laryngeal nerve: the former respond to a single shock with a short latency (3–4 ms) e.p.s.p. (Remmers, Richter, Ballantyne, Bainton & Klein, 1985) while the latter respond with a similarly short latency (up to 5 ms) i.p.s.p. (Ballantyne & Richter, 1982*a*). All neurones in the present sample were of this latter type.

The selected sample consisted of forty neurones in which the recordings remained stable for periods ranging from a few minutes up to nearly an hour and in which the inspiratory-phase membrane potential ranged from −40 mV (lower-level 'cut-off') to −70 mV, with most neurones lying between −50 and −60 mV. Of these forty neurones, thirty-three were identified as bulbospinal neurones. The remaining seven neurones showed no evidence of antidromic invasion in response to intraspinal (or vagus nerve) stimulation but were not otherwise distinguished from identified bulbospinal neurones. The sample was further divided into a set of twenty-five neurones, mostly recorded with citrate-filled micro-electrodes, in which the naturally occurring range of membrane potential patterns was established, and a set of fifteen neurones in which the responses to current or Cl^- injection were examined.

RESULTS

Membrane potential rhythm and discharge pattern

In this study neurones were classified as expiratory on the basis of the phasic character of their discharge in relation to the expiratory interval in phrenic nerve activity. Bulbospinal neurones falling into this category were very similar to one another in terms of their over-all membrane potential rhythm (hyperpolarizing during inspiration and depolarizing during expiration, conforming to the original descriptions of Salmoiraghi & Baumgarten (1961) and Mitchell & Herbert (1974)), but they were nevertheless capable of fairly extensive variation in a number of detailed features. The set of recordings shown in Fig. 1 illustrate the particular aspect of variability which was of interest in this study, namely the shape of the expiratory phase membrane potential trajectory and the manner in which threshold for discharge was approached at the start of expiration.

There are certain general features of the behaviour of these neurones which require comment. The onset of inspiration was accompanied by a rapid and usually large hyperpolarizing wave which attained maximum potential typically some time before peak discharge was developed in the phrenic nerve. This peak very often maintained a brief plateau before it began its initially rapid decay at the transition to expiration,

and during this period there occurred a more or less steep decline in potential (depolarization) (Fig. 1 *B* and *C*). The latter could, however, be replaced by a quite clear further increase in the level of membrane hyperpolarization in situations where phrenic discharge developed a sharp, late inspiratory rise in intensity (e.g. the first and third cycles in Fig. 10 *B*). Renewed hyperpolarization of this kind resulted from a renewed increase in the intensity of the inspiratory wave of i.p.s.p.s, a pattern most clearly evident after the reversal of these i.p.s.p.s by intracellular Cl^- injection

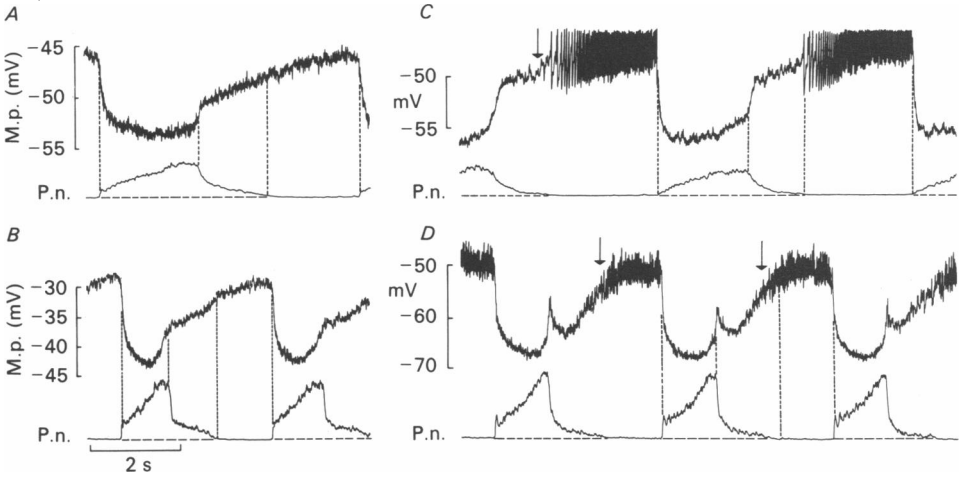

Fig. 1. Membrane potential (m.p.) pattern in a selection of expiratory bulbospinal neurones together with the corresponding records of phrenic nerve (p.n.) activity. Each record (*A–D*) is from a different neurone. The vertical lines joining the m.p. and p.n. traces in each record provide points of reference for events occurring in the phrenic nerve. The horizontal line beneath each p.n. trace in this and other Figures shows the zero-level output from the phrenic 'integrator'. The arrow on the m.p. trace of *C* points to an increase in the level of synaptic noise just prior to discharge (compare Figs. 3 *B* and *C* and 9 *A*). The arrows on the m.p. trace of *D* indicate the first spike in the expiratory burst (cf. Fig. 2 *A–C*, same neurone). All spikes attenuated in size by the pen-writer.

(Fig. 6). It was typical in these experiments that the point of inspiratory-to-expiratory transition was a readily identifiable event in the membrane potential record.

The shape of the expiratory phase membrane potential trajectory varied in an essentially continuous fashion across the sample (Fig. 1 *A–D*). While it is clear that the exact shape of this trajectory will probably depend on the average membrane potential at which it is recorded, the change in shape as it develops during the course of expiration is perhaps best seen where the spike-generating mechanism has been inactivated (Fig. 1 *A* and *B*). (The essential features of shape shown by these two examples are evident in several other figures (2 *A–C*, 3 *A–C* and 9 *A*) over a range of membrane potentials.) The variation in shape extended approximately from the kind of situation shown in Fig. 1 *A* in which expiratory depolarization developed smoothly throughout its course, unmarked by any obvious discontinuity in potential level, through an intermediate situation (Fig. 1 *B*) in which the early part of this trajectory

followed a less depolarized path than the later part with a clear transition between them, to a final state (Fig. 1*D*) in which the early part of expiration was accompanied by a distinct hyperpolarizing sag to the membrane potential.

The most prominent functional consequence of this variation in membrane potential pattern was a (variable) delay to the onset of expiratory discharge on each cycle. Delays of the magnitude shown in Fig. 1*C* and *D* were not unusual. The examples shown in Fig. 1 were selected because they introduce certain aspects of behaviour which were of a general character in these experiments, namely the tendency for neurones to show a hyperpolarizing component to the expiratory trajectory (Fig. 1*D*) or a distinct depolarizing inflexion on this trajectory (Fig. 1*B*) in the presence of a vigorous (usually fairly rapid) rhythm of phrenic discharge, or to show a smoother pattern of expiratory depolarization in the presence of a weaker (usually slower) rhythm of phrenic discharge (Fig. 1*A* and *C*; see also, Fig. 10*A* and *B*).

Under the particular conditions of these experiments, and in the absence of any experimental manoeuvre leading to a change in respiratory pattern, most neurones in the sample behaved like that of Fig. 1*C*. We are unable to attach any particular significance to the relative numbers of neurones showing one or other of these expiratory patterns, however, because individual neurones were capable of spanning virtually the entire range of patterns within a few cycles when the pattern of respiration changed (Fig. 2*A–C*) or, for instance, when water was introduced into the larynx (see Fig. 8*A*).

Phrenic nerve activity and the form of the expiratory potential trajectory

The examples shown in Fig. 1 would tend to suggest that there was no fixed relationship between the shape of the expiratory trajectory and the level of post-inspiratory activity in the phrenic nerve. However, a close relationship did emerge when the over-all level of phrenic activity was increased by running the pump at a lower rate (vagi cut) and allowing end-tidal CO_2 to rise to the upper end of the range usual in these experiments (close to 5 vol. %). In one case this had the effect of introducing a fair amount of variability in the level of post-inspiratory phrenic discharge. The effect of this variability on membrane potential trajectory is shown in Fig. 2*A–C* (same neurone as in Fig. 1*D*).

Fig. 2*A* shows a sequence, shortly after impalement, in which on some cycles phrenic discharge declined rapidly at the cessation of inspiration. On these particular cycles the onset of expiratory depolarization was brisk, quickly reaching threshold for discharge so that there was essentially no delay to the onset of the expiratory burst. Fig. 2*B* shows the more typical phrenic pattern, obtained about half a minute later, when there was a well developed phase of post-inspiratory activity and an associated hyperpolarizing sag to the early (post-inspiratory) component of the expiratory potential trajectory. Not unexpectedly, the onset of the expiratory burst was delayed compared with most cycles in Fig. 2*A*. The sequence shown in Fig. 2*C* was recorded between that of 2*A* and *B* and illustrates the process of transition from one pattern to the other. On successive cycles in Fig. 2*C* the intensity of the post-inspiratory component of phrenic activity became progressively greater. This increase was accompanied by a gradual change in the shape of the potential trajectory from a steeply rising depolarization which quickly reached threshold (first cycle),

through an intermediate development of post-inspiratory hyperpolarization and increasingly delayed onset to expiratory discharge (second and third cycles), to a final state (fourth cycle) in which the rapid onset to post-inspiratory hyperpolarization resulted in the latter attaining nearly the same hyperpolarized membrane potential

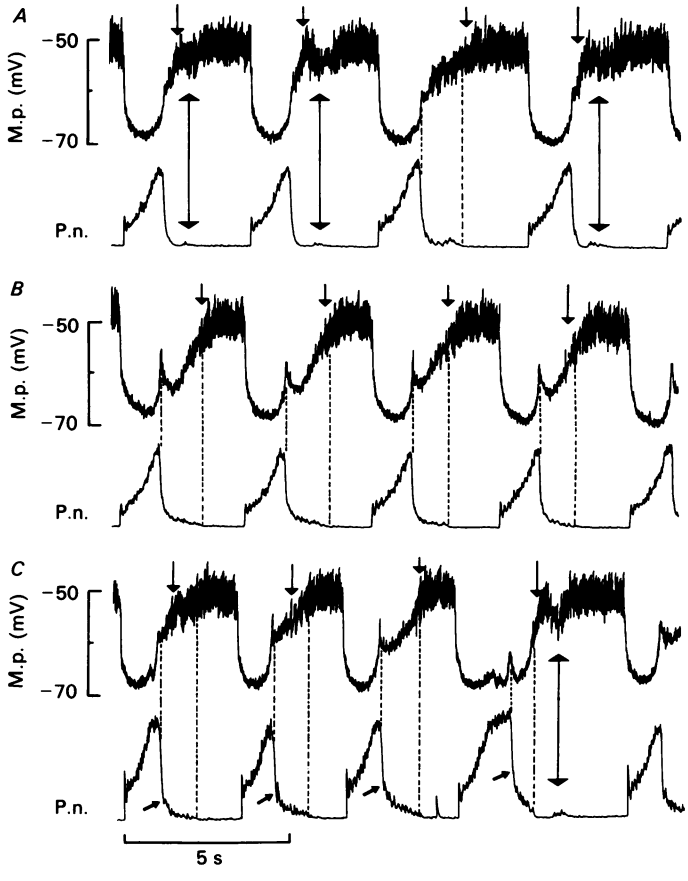

Fig. 2. Influence of variation in the time–intensity profile of post-inspiratory phrenic nerve (p.n.) activity on the membrane potential (m.p.) trajectory (same neurone shown in Fig. 1 *D*). The time constant of the phrenic 'integrator' was shortened in *C* to emphasize the changing profile (diagonal arrows) of post-inspiratory activity. Where this activity was present, its duration is indicated by the lines joining the p.n. and m.p. traces. The downward-pointing arrows on the m.p. trace of *A–C* indicate the first spike in the augmenting component of the expiratory burst. A single spike was occasionally discharged immediately following release from inspiratory hyperpolarization (e.g. second and third cycles of *C*). All spikes are attentuated in this pen-writer record. For explanation of double-headed arrows see text.

as that reached during the inspiratory ramp in the phrenic nerve. On returning the pump to its original rate, the respiratory rhythm gradually slowed down (Fig. 10*A*, same neurone) and although a post-inspiratory pattern of hyperpolarization was still clearly evident, the precision with which it followed the time course of post-inspiratory phrenic activity was somewhat diminished.

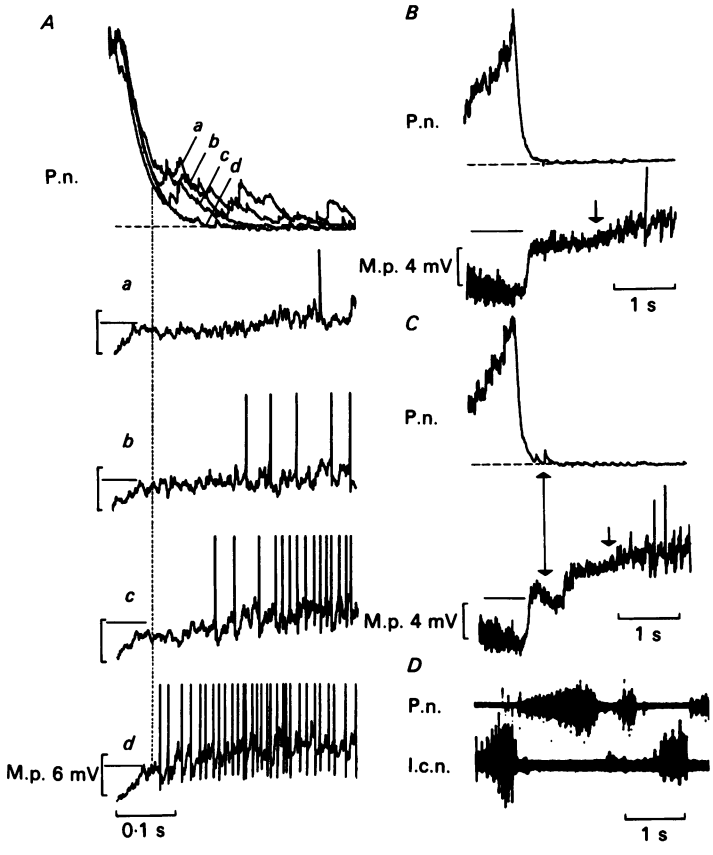

Fig. 3. *A*, integrated profiles of post-inspiratory phrenic nerve (p.n.) activity at different levels of intensity (*a–d*) and corresponding membrane potential trajectories (m.p., *a–d*). The horizontal bar at the start of each m.p. trace is a reference membrane potential of −60 mV. *B* (same format as *A*), shows an initially flat and (arrow) later depolarizing potential trajectory preceding the first spike. *C* (same neurone as *B*), shows a brief post-inspiratory discharge in the phrenic nerve and accompanying hyperpolarization in the membrane potential (double-headed arrow). The downward pointing arrow later on the m.p. trace again signifies a change in intensity of expiratory synaptic noise (cf. *B*). The horizontal bar in the m.p. traces of *B* and *C* is a reference membrane potential of −50 mV. The high level of inspiratory i.p.s.p. noise in *B* and *C* was due to high frequency synchronization of i.p.s.p.s which was present in three neurones in the sample (80–100 Hz frequency) (see also, Cohen, 1979; Mitchell & Herbert, 1974). The varying size of spikes in *A–C* was due to the digitizing procedure which displayed only portions of each spike at these sweep speeds. *D*, simultaneous record from the phrenic nerve and a filament of the T5–6 internal intercostal nerve (i.c.n.) (vagi cut) from Richter (1982). Note similar relationship to that shown on the fourth cycle of Fig. 2*C*.

It should be emphasized in connexion with the sequence *A*, *C*, *B* in Fig. 2 that pattern changes of this kind occurred several times at this elevated end-tidal CO_2. We do not suggest that the intensification of post-inspiratory phrenic activity was specifically related to CO_2 but that, in this case, it was related to recurring changes in the pattern of respiration. A possibly rather specific effect of ventilation in

considerably lengthening post-inspiratory activity and depressing inspiratory activity in the phrenic nerve was produced by reducing the pump rate to levels at which hypoxic effects may have developed. It was not possible to follow the pattern of membrane potential changes in these cases because the associated increase in blood pressure resulted in the cells being 'lost'.

Where post-inspiratory phrenic activity was present, its 'retarding' effect on the timing of expiratory discharge was qualitatively the same from neurone to neurone, but the sensitivity of the membrane potential trajectory to this activity was variable. Fig. 3*A* shows a situation in which a vigorous pattern of expiratory discharge was modulated by even quite moderate fluctuations in the level of post-inspiratory activity occurring irregularly during the recording. Note also in Fig. 3*A* that the initial rate of decay of phrenic activity (up to the vertical dotted line) was about the same in each case and, correspondingly, that up to this point there was no significant difference in membrane potential pattern between traces.

The relationship which emerges from Figs. 2*A–C* and 3*A* refers to the commonest situation, i.e. where some variable level of post-inspiratory phrenic activity was present. It does not follow, however, that where this activity was absent (or noticeably weak) the expiratory burst necessarily began early. This was not the case, though long delays in the absence of post-inspiratory phrenic activity were associated with very slow rates of respiration (*ca.* 8/min in Fig. 3*B*). However, even in cases of this sort, the occasional appearance of post-inspiratory activity, e.g. as an isolated instance in Fig. 3*C*, 'exerted' a distinct hyperpolarizing action on the membrane potential.

These observations suggest an inhibitory effect of post-inspiratory phrenic activity on expiratory neurones. This may also be inferred from another feature of Fig. 2*A* and *C*, namely the occurrence of a transient hyperpolarizing wave in the membrane potential on the appearance of even a minute phasic burst in the phrenic nerve during the course of expiration (double-headed arrows). These bursts are of interest because both in terms of their time of occurrence relatively early in expiration and their association with a hyperpolarizing wave in the membrane potential, they resemble a residue of the normal, gradually declining pattern of post-inspiratory discharge whose initial component was presumably suppressed on those particular cycles. The consequences of this suppression for membrane potential pattern are those described earlier. This interpretation is perhaps most plausible in relation to the fourth cycle shown in Fig. 2*C*.

A similar pattern of temporal relationships to that shown by the fourth cycle of Fig. 2*C* is also sometimes encountered in the discharge recorded from phrenic and internal (expiratory) intercostal nerves (Fig. 3*D*; see also Bainton, Kirkwood & Sears, 1978, their fig. 10*A*).

Post-inspiratory synaptic inhibition

On the basis of the observations described above, our expectation was that the shape of the expiratory phase membrane potential trajectory is determined at least partly by the presence of i.p.s.p.s. Fig. 4 shows the behaviour of one of five neurones ($n = 15$) in which post-inspiratory i.p.s.p.s occurred spontaneously. Fig. 4*A* shows the membrane potential pattern obtained shortly after penetration with a KCl-filled

micro-electrode. In this, as in some other instances where low-resistance micro-electrodes (Methods) were employed to ensure adequate entry of Cl^-, the process of i.p.s.p. reversal began virtually immediately on penetration (see below). This process was accelerated when Cl^- was actively injected, and Fig. 4*B* shows the membrane potential response to the injection of a steady hyperpolarizing current (−12 nA) at *ca.* 3 min following the start of injection.

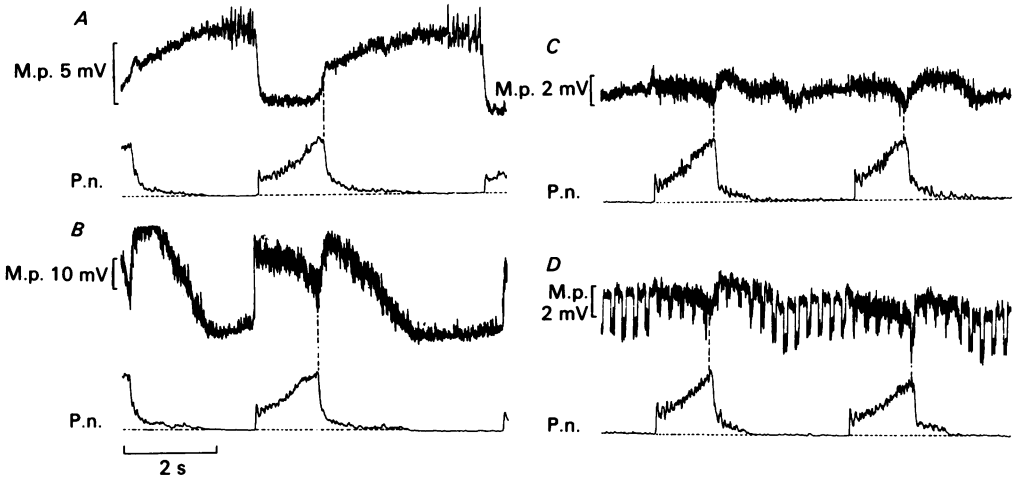

Fig. 4. Post-inspiratory inhibition. *A*, membrane potential (m.p.) and phrenic nerve (p.n.) patterns recorded on penetration. Only a few control cycles were obtained before i.p.s.p. reversal by leakage of Cl^- into the cell became clearly evident. *B*, membrane potential response to hyperpolarizing d.c. current (−12 nA) showing depolarizing reversal of inspiratory and post-inspiratory i.p.s.p.s. *C*, membrane potential pattern following Cl^- injection (*ca.* 50 nA min; current off at this time). *D*, temporal pattern of hyperpolarizing voltage responses to a train of constant current pulses (−0·7 nA, 40 ms) (no d.c. current). Maximum resistance at the expiratory-to-inspiratory transition ranged from 2·5 to 3·3 MΩ, and minimum during the two i.p.s.p. waves was *ca.* 1 MΩ.

There were two prominent effects of current at this intensity. First, the inspiratory hyperpolarizing wave (Fig. 4*A*) was reversed to a depolarizing wave (Fig. 4*B*), while the depolarizing shift in potential at the cessation of inspiration (Fig. 4*A*) was converted to a hyperpolarizing shift (Fig. 4*B*). This pattern of reversal of inspiratory i.p.s.p.s was repeated from cell to cell in the presence of comparable patterns of phrenic discharge. The second effect was to convert the rather slowly developing pattern of expiratory depolarization (Fig. 4*A*) to a rapid onset depolarizing wave whose subsequent decline in amplitude followed about the same time course as that of post-inspiratory phrenic activity (Fig. 4*B*). In the expectation that this post-inspiratory depolarizing wave was also due to Cl^--dependent i.p.s.p.s, injection was continued sufficiently long (*ca.* 50 nA min in this case) to shift the i.p.s.p. reversal potential in a depolarizing direction, and the current was then turned off (Fig. 4*C* and *D*). Under these conditions both the inspiratory and post-inspiratory waves were relatively depolarizing and accompanied by a high level of synaptic noise (see also Fig. 5*A* and *B*).

Fig. 4*D* shows the pattern of voltage responses of the cell to the injection of small hyperpolarizing constant current pulses. The amplitude of these responses was greatest shortly after the cessation of post-inspiratory phrenic activity and smallest during the wave of depolarizing inspiratory i.p.s.p.s. The voltage response increased in size briefly at the inspiratory-to-expiratory transition and then again showed a reduction during the post-inspiratory wave of depolarization. The magnitude of this latter reduction followed closely the corresponding time–intensity profile of both the post-inspiratory synaptic wave and post-inspiratory discharge in the nerve. Behaviour of this kind implies the arrival of a wave of Cl^--dependent i.p.s.p.s which begins abruptly at the transition to expiration, declines in intensity as expiration proceeds and which is separated by a distinct, if brief, pause from the preceding inspiratory wave of i.p.s.p.s.

In Fig. 4*A* the shape of the membrane potential pattern was almost certainly modified by partial i.p.s.p. reversal related to Cl^- leakage on penetration. We frequently observed in cases of this sort (including observations made on inspiratory neurones) that if the cell was successfully 'held', recovery was rapid. The integrity of the cell manifested itself in an increase in membrane potential and (if Cl^- was not injected) in a restoration of i.p.s.p. polarity. This last may be related to the fact that while both inspiratory and post-inspiratory i.p.s.p.s were fairly readily reversed by moderate levels of Cl^- injection, this reversal was usually not maintained beyond 1–2 min when the current was turned off, and both patterns of i.p.s.p.s exhibited a continuous, if partial, recovery to their hyperpolarizing state. This suggests the capacity for extrusion of at least part of the additional (injected and leaked) Cl^-. Repeated, small injections of Cl^- resulted in a stable pattern of depolarizing i.p.s.p.s (no current injection) at least over periods of minutes (cf. Faber & Korn, 1982). The increased synaptic noise level shown in Fig. 5*A–C* compared with Fig. 4*C* resulted from this procedure.

Factors influencing the occurrence of post-inspiratory i.p.s.p.s

Pattern of respiration. Post-inspiratory i.p.s.p.s were spontaneously present only where there was a strong *inspiratory* component to phrenic discharge. Under these conditions, fluctuations in the intensity or duration of *post-inspiratory* activity were accurately reflected in the time–intensity profile of post-inspiratory i.p.s.p.s (Fig. 4*B–D*). Where the over-all pattern of phrenic discharge was weak, e.g. at high pump rates and reduced end-tidal CO_2, these i.p.s.p.s were weak or absent. In the case of the sequence shown in Fig. 5*A–C* (same neurone as in Fig. 4), retrospective examination of the anaesthetic protocol pointed to a supplementary injection as the most probable cause of the slowing of the respiratory rhythm and decrease in phrenic amplitude. At the stage reached in Fig. 5*C*, at which post-inspiratory i.p.s.p.s were no longer detectable, a steady hyperpolarizing current was injected at an intensity which was usually sufficient to reveal even a weak i.p.s.p. input, but there was no evidence of post-inspiratory i.p.s.p.s (Fig. 5*D*). This injection, with consequent complete reversal of inspiratory i.p.s.p.s, also provided a check against the possibility that the gradual 'loss' of post-inspiratory i.p.s.p.s in the sequence *A–C* was not simply due to a coincident redistribution of intracellular Cl^-. Similarly, the injection of a train of hyperpolarizing current pulses (Fig. 5*E*) revealed that resistance increased

immediately at the transition to expiration and remained high throughout expiration. We conclude that post-inspiratory i.p.s.p.s were absent under these conditions.

Shortly after the sequence of events shown in Fig. 5, the pattern of post-inspiratory i.p.s.p.s was restored in this neurone by a period of stimulation of the superior laryngeal nerve (Fig. 6). The record in Fig. 6 thus begins with inspiratory i.p.s.p.s in their reversed (depolarizing) state, maintained by application of hyperpolarizing

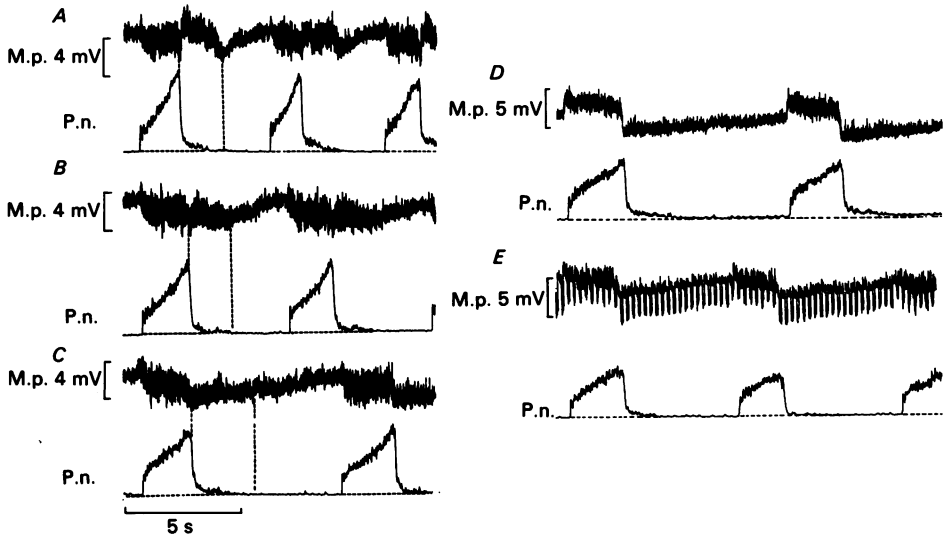

Fig. 5. Same neurone as in Fig. 4 showing gradual slowing of the respiratory rhythm (see text) and weakening of post-inspiratory i.p.s.p.s. *A–C*, records taken at about 30 s intervals. No current injection during this period but the records were obtained after a further period of Cl^- injection (10 nA . min) following Fig. 4*C* and *D*. The vertical lines indicate duration of post-inspiratory phrenic activity. *D*, response to hyperpolarizing d.c. current (*ca.* −1·5 nA). *E*, temporal pattern of hyperpolarizing voltage responses to a train of constant-current pulses (−1·9 nA, 40 ms) obtained *ca.* 5 min after response shown in *D*.

d.c. current (*ca.* −4 nA). There were two main effects of stimulation. The plateau-like pattern of phrenic discharge which preceded the stimulus was converted to a ramp-like pattern, an effect which became most prominent when the stimulus was turned off and which continued to develop on succeeding cycles for a short period. Correspondingly, there occurred a change in the time–intensity profile of inspiratory depolarizing i.p.s.p.s which developed a late peak in their intensity. The second effect consisted in the gradual re-emergence of a post-inspiratory depolarizing wave which, again, became most pronounced shortly after stimulation, which is also when the ramp-like development of phrenic activity was at its most intense. There was no correspondingly large increase in post-inspiratory phrenic activity. That the stimulus-evoked post-inspiratory depolarizing wave was indeed due to i.p.s.p.s was verified by passing a steady depolarizing current into the cell which re-reversed both inspiratory and post-inspiratory i.p.s.p.s (Fig. 7*A* and *B*). Note that in Fig. 7*A* the development of post-inspiratory i.p.s.p.s following superior laryngeal nerve stimulation had

progressed to a stage where they were larger than the wave of inspiratory i.p.s.p.s. Subsequently they diminished considerably in intensity (see Fig. 10*D*).

Adequate stimulation of laryngeal receptors. The comparatively slow time course in the emergence (Fig. 6) and decline (Fig. 5*A–C*) of post-inspiratory i.p.s.p.s would tend to suggest that their occurrence was directly related to the over-all change in pattern of respiration, consistent with our general impression. However, adequate stimulation of 'irritant' (but possibly also other) laryngeal receptors was capable of evoking these

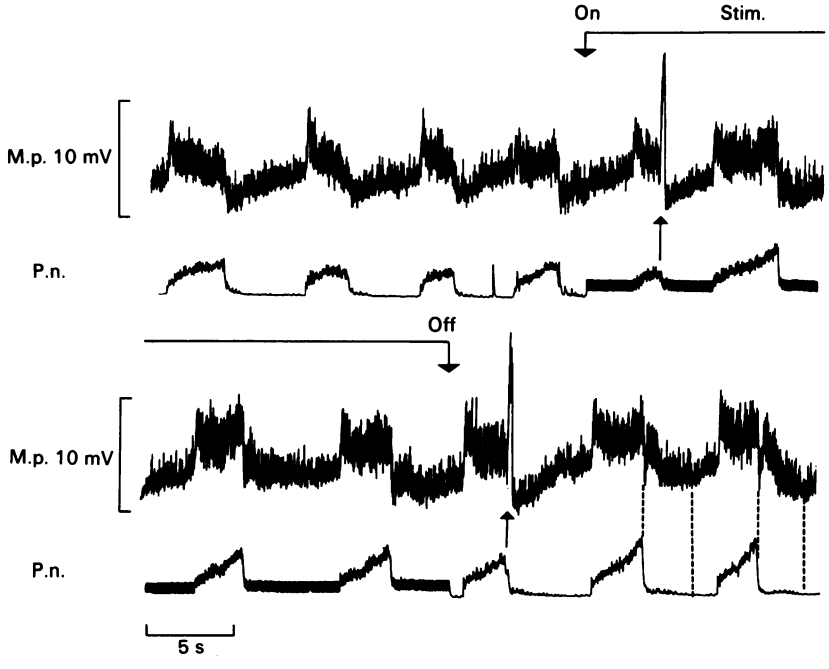

Fig. 6. Reflexly elicited post-inspiratory i.p.s.p.s in the same neurone shown in Figs. 4 and 5. Membrane potential (m.p.) pattern and phrenic nerve (p.n.) activity prior to, during and immediately following stimulation (stim.) of the superior laryngeal nerve (3·3 V; 0·05 ms; 20 cycles/s). Inspiratory i.p.s.p.s maintained in a depolarizing state by a steady injection of hyperpolarizing current (*ca.* −4 nA). The vertical lines joining the p.n. and m.p. traces indicate estimated time course of post-inspiratory i.p.s.p.s. Note increasing amplitude of post-inspiratory i.p.s.p.s on the last two cycles (cf. Fig. 7*A*). The two large depolarizing transients (arrows) are associated with a 'notch' on the decaying phase of the phrenic record (only clearly visible on the lower record). Further explanation of this transient is given in connexion with Fig. 10.

i.p.s.p.s in a manner that was unconnected with any *consistent* direction of change in either inspiratory or post-inspiratory components of phrenic activity. This effect (Fig. 8) was elicited in seven of ten neurones tested which initially showed no evidence of these i.p.s.p.s.

The traces labelled *a* in Fig. 8*A*1 and 2 show the integrated phrenic nerve records and corresponding membrane potential trajectories shortly before the introduction of water into the larynx. The traces labelled *b* show these features on the first (*A*1) and third (*A*2) cycles as water was injected. The main effect of the stimulus was

to produce a distinct hyperpolarizing sag to the post-inspiratory component of the membrane potential trajectory and to change the frequency characteristics of the synaptic noise (particularly evident in *A* 2). The arrows in *a* (*A* 1 and 2) point to the first spike in the expiratory burst prior to stimulation. The timing of this first spike was delayed by several tenths of a second (beyond the time scale of the trace)

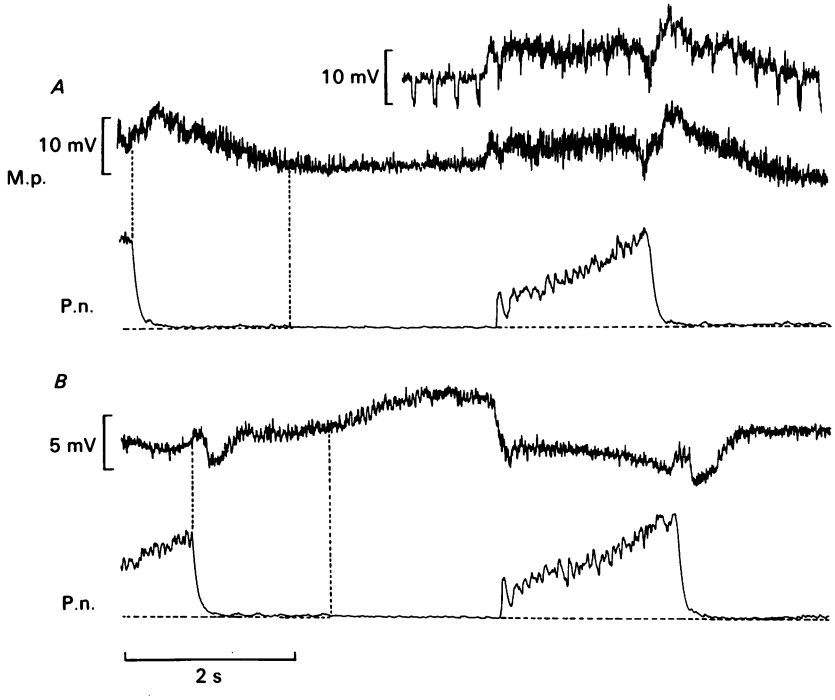

Fig. 7. Evidence that the reflexly elicited post-inspiratory depolarizing wave shown in Fig. 6 contains a large i.p.s.p. component. Same neurone as in Fig. 6 but records obtained a few cycles after cessation of superior laryngeal nerve stimulation. *A*, depolarizing inspiratory and post-inspiratory i.p.s.p.s during hyperpolarizing current injection, adjusted exactly to −4·0 nA. The inset record shows a fairly progressive increase in input resistance during the course of the post-inspiratory wave (tested with a train of hyperpolarizing current pulses, −2·2 nA, 40 ms). *B*, re-reversed inspiratory and post-inspiratory i.p.s.p.s by steady depolarizing current (+4·0 nA). The difference in time course of the post-inspiratory synaptic wave in *A* and *B* may be due to interaction with a large excitatory input or to i.p.s.p. components with slighly different reversal properties. The vertical lines joining p.n. and m.p. traces indicate duration of very low level of post-inspiratory phrenic activity (checked against the original activity on the oscilloscope).

following water injection. While this evoked post-inspiratory hyperpolarization extended over about the same time course as post-inspiratory activity in the nerve, the latter did not change appreciably (in this instance) as a result of the stimulus (Fig. 8*A*1 and 2). On some occasions it was reduced in intensity or suppressed (e.g. Fig. 8*B* and *C*). That this post-inspiratory hyperpolarization was due to a wave of i.p.s.p.s is shown, for another neurone, in Fig. 8*B* and *C* where they were reversed by a maintained injection of (Cl^-) current.

The two principal inferences which we draw from these observations are, first, that the relationship depicted for example in Fig. 4 between post-inspiratory i.p.s.p.s and post-inspiratory phrenic activity may vary with the depth of anaesthesia, and secondly, that post-inspiratory i.p.s.p.s (and consequently the shape of the expiratory potential trajectory) may be controlled by activity in laryngeal afferents, at least to some extent independently of events occurring in the phrenic nerve.

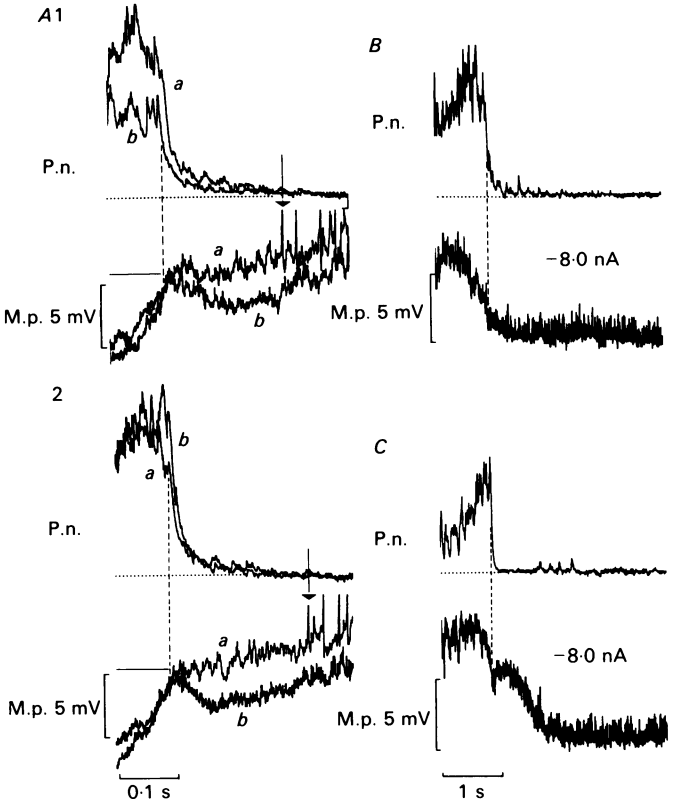

Fig. 8. Reflex activation of post-inspiratory i.p.s.p.s by adequate stimulation of laryngeal receptors. *A* 1 and *A* 2 (same neurone), membrane potential (m.p.) and phrenic nerve (p.n.) patterns prior to (*a*) and during (*b*), injection of water into the larynx. The vertical dashed line indicates the point of inspiratory-to-expiratory transition. The horizontal bar at the start of each m.p. record is a reference membrane potential of −45 mV. Spikes (present only in traces labelled *a*) are digitized and not full size. *B* and *C* show a different neurone with reversed i.p.s.p.s in the presence of a hyperpolarizing d.c. current. *B* shows the pattern prior to, and *C* the pattern during, the injection of a weakly alkaline solution into the larynx. Note depolarizing post-inspiratory i.p.s.p.s in *C*.

Differences in synaptic behaviour in early and late expiration

Fig. 9 *A–D* shows the behaviour of one of ten neurones which failed to show clear evidence of spontaneous post-inspiratory i.p.s.p.s (comparable to those of Fig. 4) but in which the membrane potential response to current injection and stimulation of the superior laryngeal nerve revealed differences in synaptic behaviour in early

(post-inspiratory) and late expiration. In the example of Fig. 9*A* the control pattern of expiratory depolarization was steep but in some others showing the same general kind of response to current or Cl^- injection it was flatter (e.g. Fig. 1*C*). Fig. 9*B* shows the membrane potential response to the passage of a steady hyperpolarizing (Cl^-) current (−8·0 nA). The wave of inspiratory i.p.s.p.s was reversed in typical fashion and showed a similar time–intensity profile to that of the control pattern. At this

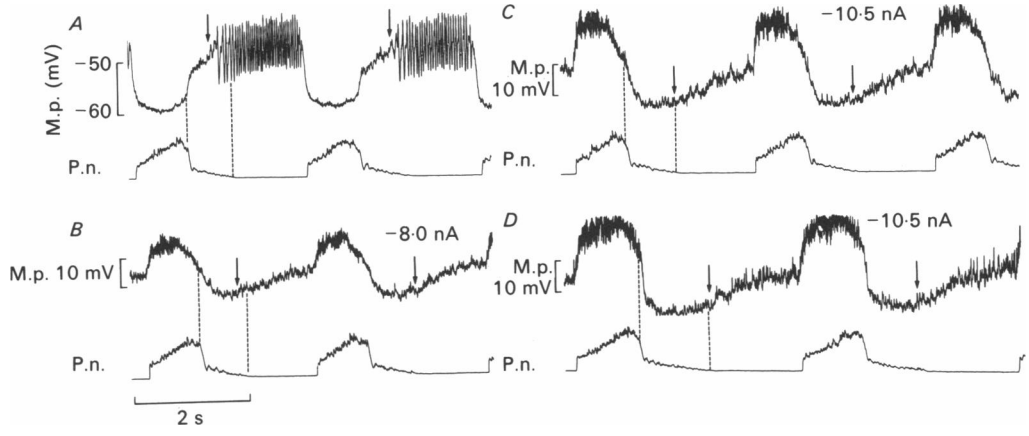

Fig. 9. Differential synaptic behaviour during post-inspiratory and later stage of expiration. *A*, control membrane potential (m.p.) and phrenic nerve (p.n.) patterns. *B* and *C* show the effect of hyperpolarizing d.c. current at the values shown. *D*, membrane potential pattern (same conditions as *C*) immediately after cessation of superior laryngeal nerve stimulation (2·8 V; 0·05 ms; 35 cycles/s for 40 s). The dashed lines linking p.n. and m.p. traces show duration of post-inspiratory phrenic activity and its relationship to changes (arrows) in the level of expiratory synaptic noise.

current intensity (Fig. 9*B*) the point of inspiratory-to-expiratory transition was not well defined in the membrane potential record (in contrast to the situation described earlier for control recordings from these neurones) but instead there occurred a gradual repolarization which began shortly before inspiration ceased and extended into the early part of expiration. One interpretation of this repolarization and reduction in the level of synaptic noise is that it reflects a continuous diminution in i.p.s.p. intensity, beginning towards the end of inspiration and continuing on into expiration. A reduction in i.p.s.p. noise level might also occur if the transition from inspiration to expiration is accompanied by a change in the population of active inhibitory synapses to a more remote location on the cell.

On increasing current intensity to −10·5 nA (Fig. 9*C*), the amplitude of the depolarizing wave of inspiratory i.p.s.p.s increased as expected, and the inspiratory-to-expiratory transition became more clearly defined as a relatively rapid phase of membrane repolarization, implying that the shunting effect of inspiratory i.p.s.p.s is to a large extent removed at this point (disinhibition). The hyperpolarizing effect of injected current was greatest during the early part of expiration and increased in a graded fashion as current strength was increased (Fig. 9*B* and *C*). This latter does not necessarily disprove the occurrence of post-inspiratory i.p.s.p.s (which, as

indicated above, might be generated at sites electrically remote from the current source) but it does suggest the possibility that the low level of synaptic noise accompanying early expiration in Fig. 9*B* and *C* was due to a low level of e.p.s.p. input (disfacilitation) (see Discussion). It should also be noted that in neurones where post-inspiratory i.p.s.p.s were clearly present they appeared to show about the same 'sensitivity' to injected Cl^- as the readily reversed inspiratory i.p.s.p.s. As expiration progressed the hyperpolarizing action of current diminished and the post-inspiratory plateau-like potential gave way to a noisier, incrementing pattern of synaptic depolarization. This transition is more evident on the second than on the first cycle of Fig. 9*B*, a feature which illustrates the variable sharpness which was also evident in control recordings from these neurones (compare the first and second cycles of Fig. 1*C*). An increase in the strength of applied current brought out the distinction more clearly (Fig. 9*C*).

This distinction was also emphasized, at least for a few cycles, following a period of stimulation of the superior laryngeal nerve. Three features may be noted in the post-stimulus behaviour shown in Fig. 9*D*. First, both the rate and final amplitude of the hyperpolarizing shift in potential at the cessation of inspiration were increased in Fig. 9*D* compared with 9*C* (same current strength), an effect which was probably partly due to a more abrupt termination of the intensified pattern of inspiratory i.p.s.p.s which followed laryngeal stimulation in this as in other neurones in the sample (Figs. 6 and 8*B* and *C*). Secondly, the expiratory interval was lengthened for a short time by a nearly proportional increase in the duration of the post-inspiratory component of activity in the phrenic nerve and in the duration of the subsequent period of silence in the nerve. The third feature was that this change in expiratory duration was accompanied by an increase in duration and amplitude of post-inspiratory hyperpolarization and with an increase in the level of synaptic depolarization and noise in the later stage of expiration. Note also on the second cycle of Fig. 9*D* that this difference in the synaptic character of early and late expiration persisted as the expiratory interval again shortened. These observations suggest that a major effect of stimulation was to intensify the normally prevailing, if often small, difference in the character of synaptic activity during each stage.

While it is possible that the reversal of inspiratory i.p.s.p.s might initiate secondary effects, e.g. activation of one or more K^+ conductances, which could contribute to post-inspiratory hyperpolarization, precisely comparable effects to those shown in Fig. 9 were seen under conditions of only partial i.p.s.p. reversal. Moreover, the rapid component to this hyperpolarization in Fig. 9 began only after inspiration ceased when the membrane was already partially repolarized by the decline in i.p.s.p. intensity. Finally, the observed relationship of this hyperpolarization to the changing time course of post-inspiratory phrenic activity (Fig. 9*C* and *D*) argue in favour of an interpretation based on synaptic rather than non-synaptic mechanisms. In situations where the amplitude of phrenic discharge was low, usually associated with a slow respiratory rhythm, the 'two-stage' pattern of expiratory synaptic activity of the kind shown in Fig. 9 was absent.

A variant on the post-inspiratory pattern of inhibition

Fig. 10*A* and *B* shows a variant on the pattern of post-inspiratory inhibition which was observed on a number of occasions. This consisted in the appearance of a prominent hyperpolarizing transient on the occurrence of a brief but quite clear renewel of phrenic activity on the decaying phase of the integrated record (double-headed arrows in *A* and *B*). From cell to cell this large transient was readily

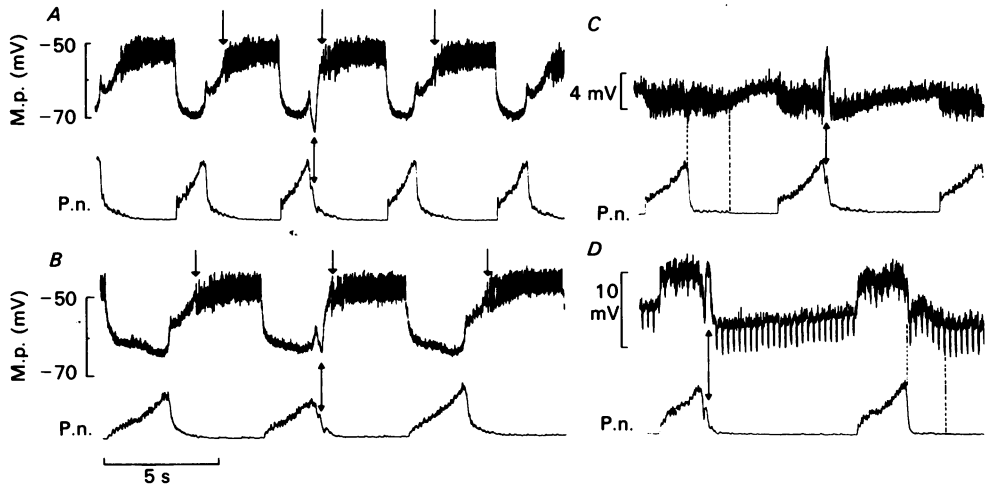

Fig. 10. A variant on the post-inspiratory pattern of inhibition. *A* and *B*, membrane potential (m.p.) and phrenic nerve (p.n.) patterns recorded under control conditions. (*A* is the same neurone shown in Fig. 1*D*). The downward-pointing arrows on each m.p. trace indicate the time of onset of expiratory discharge (spikes attenuated by the pen-writer). The double-headed arrow indicates the relationship of the hyperpolarizing transient to a 'notch' on the integrated phrenic record. *C* shows in a different neurone reversed (depolarizing) inspiratory and post-inspiratory i.p.s.p.s and reversal of the hyperpolarizing transient shown in *A* and *B*. (No current injection in *C*; record obtained from about the same period as that shown in Fig. 5*B*.) *D* shows the voltage responses of the cell to a train of hyperpolarizing current pulses (−2·2 nA, 40 ms), superimposed on a d.c. current (−4·0 nA). Record obtained some minutes after that of Fig. 7. Note in *C* and *D* there was no evidence of further significant i.p.s.p. activity in expiration following the occurrence of the large inhibitory transient (cf. Fig. 6).

identifiable by its relationship to this renewed burst of activity which appeared as a 'notch' in the phrenic record. The transient began about 50–150 ms before the 'notch'. Following Cl^- injection this hyperpolarization was converted to a depolarizing potential (Fig. 10*C* and *D*, different neurone), from which we conclude that it was generated by a wave of i.p.s.p.s. The inhibitory transient was a spontaneous event in the two examples of Fig. 10*A* and *B*, but Fig. 6 shows an example of the more general observation in these experiments, namely that transients of this kind were most common during, and sometimes following, stimulation of the superior laryngeal nerve. (In Fig. 6 these transients (arrows) appear as reversed (depolarizing) i.p.s.p. waves in the presence of a steady hyperpolarizing current.)

On the basis of its timing it seems likely that this inhibitory wave represents an

unusually short-lasting and intense wave of post-inspiratory i.p.s.p.s. Whenever it occurred, the 'normal' pattern of expiratory depolarization (defined in relation to the other cycles in the sequence) was replaced on that one cycle by a more rapid depolarization and advanced onset to expiratory discharge (compare the membrane potential trajectory and time of onset of the expiratory burst (arrows) on the preceding and succeeding cycles in Fig. 10*A* and *B*). Although it is possible that other (non-synaptic) mechanisms are involved in this temporary alteration in behaviour, we would suggest that the change in shape of the expiratory potential trajectory is largely explained by this changed pattern of post-inspiratory i.p.s.p.s. Note in Fig. 10*C* and *D* that following the large i.p.s.p. transient there was no evidence of further significant i.p.s.p. activity during the course of expiration.

DISCUSSION

In this study we have shown that the expiratory-related synaptic activity of expiratory neurones does not, in general, present a uniform appearance throughout its time course. The changing character of this activity manifested itself in a variable delay to the onset of the expiratory burst. In turn, the burst was preceded by a membrane potential pattern which varied from steeply depolarizing, through a plateau-like potential, to a distinct hyperpolarizing potential. The questions of particular interest then, reduce to the mechanisms responsible for this variation and whether they can be accommodated by what is currently known about the supraspinal organization of respiratory neurones.

In general terms, the time at which an action potential is discharged may be expected to depend on a number of factors, including average membrane potential, the amplitude and frequency characteristics of synaptic events and relatively longer lasting effects of a variety of non-synaptic conductances (Bryant & Segundo, 1976; Knox & Poppele, 1977; Kirkwood & Sears, 1978; Kirkwood, 1979; Fetz & Gustafsson, 1983; Crill & Schwindt, 1983). These factors may be presumed to apply to the present case where, in a given neurone under unchanging intracellular recording conditions, the time course of events preceding the expiratory burst varied from something less than *ca.* 50 ms to more than 1 s beyond the point at which inspiratory i.p.s.p.s ceased. With variation operating over this range, it is clear that the *exact* point in expiration at which discharge began in these recordings has no special significance. There is thus no suggestion in these results that the expiratory interval is inflexibly divided into a post-inspiratory phase of 'silence' and a later phase of discharge. Such a pattern may sometimes be the case but the character of the expiratory trajectory was such as to suggest a continuous gradation in the extent to which the post-inspiratory and the later stage of expiration are synaptically differentiated.

Our use of the term 'post-inspiratory' in connexion with the membrane potential pattern assumes a certain kind of relationship, at least in the first instance, to post-inspiratory activity in the phrenic nerve (see also Ballantyne & Richter, 1984). This usage is, we believe, justified by their parallel variation in the presence of a strongly developed phrenic rhythm and the relationship is one which, with certain exceptions described in the Results, is preserved in relation to specific synaptic features associated with this part of the expiratory interval. The fact that this

relationship is not invariant but may change with depth of anaesthesia or the influence of activity in laryngeal afferents implies that the relationship is not causal and certainly complicates the issue of arriving at any general hypothesis for the special character of this part of the respiratory cycle. The usefulness of distinguishing the early part of expiration in some way from the rest of the cycle does not depend, however, on its having a certain relationship to detailed features of the respiratory motor output, even if a particular kind of relationship is *usually* observed, as in the present case. The question is really one as to whether the connectivity of the respiratory network is such that following the cessation of inspiration it adopts for a short time a set of relationships which are different from those occurring later in expiration. In some experimental conditions these changing relationships may be reflected fairly directly in phrenic nerve activity; in other conditions they may not.

We interpret the present results in terms of the operation of 'synaptic mechanisms', i.e. in terms of neuronal connectivity, with the above ideas in mind. It is nevertheless clear that mechanisms intrinsic to expiratory neurones might contribute to generating the observed delays to discharge. The operation of such mechanisms would not, however, constitute a distinct *phase* of the cycle in the sense implied above. Their full discussion is beyond the scope of this paper but some consideration of the extent to which the results may have been influenced by non-synaptic mechanisms is relevant to the present discussion and is briefly dealt with below.

In principle, activation of a K^+ conductance would provide a means of extending the effective inhibitory period in these neurones beyond the point at which Cl^--dependent inspiratory i.p.s.p.s cease. Two types of conductance mechanism would seem particularly relevant to the present case of delaying the onset of expiratory discharge: (1) a Ca^{2+}-dependent K^+ conductance activated by the rapid depolarization which occurs at the inspiratory-to-expiratory transition. Expiratory bulbospinal neurones are equipped with a conductance(s) of this kind, but it would appear not to be significantly involved in shaping the post-inspiratory membrane potential trajectory since intracellular injection of the Ca^{2+} chelator EGTA in quantities sufficient to considerably reduce a post-tetanic hyperpolarization in these neurones (Mifflin, Ballantyne, Backman & Richter, 1985; S. Mifflin, D. Ballantyne & D. W. Richter, unpublished data) exerts no obvious effect on the form of this trajectory; (2) a K^+ conductance of A-type. A-currents have been demonstrated or their presence inferred in numerous types of neurones, including what may be respiratory neurones in slice preparations (Dekin & Getting, 1984; J. Champagnat & D. W. Richter, unpublished data). Moreover, the form of the membrane potential rhythm which typifies expiratory neurones would seem ideally suited to establishing the conditions required for A-current activation at the transition to expiration. It seems quite likely that under the appropriate membrane potential conditions, a mechanism of this type may be involved in delaying expiratory discharge. It should be noted, however, that in the present experiments the 'two-stage' pattern to the expiratory membrane potential trajectory persisted independently of whether it was preceded by hyperpolarizing or depolarizing inspiratory i.p.s.p.s. While it is clear that weak effects would probably not have been detected in the present experiments, it seems likely that these conductances play a relatively subordinate role to the control exerted by synaptic input.

Synaptic differentiation of the expiratory interval

At one extreme, there was no obvious differentiation and expiratory synaptic noise and membrane potential changed in a quite smooth manner, not unlike the trajectories often seen in spinal respiratory motoneurones (Sears, 1964; Berger, 1979; Kirkwood, Sears, Tuck & Westgaard, 1982). More usually in these experiments, however, there was evidence of a more or less distinct change in synaptic noise or rate of depolarization, or both, at some point in expiration. The distinction was usually evident simply from inspection of the control membrane potential trajectory but it was more clearly brought out as one of the (post-stimulus) effects of superior laryngeal nerve stimulation; e.g. in Fig. 9*D* the effect of stimulation appeared to be one of exaggerating pre-existing differences between the post-inspiratory and later components of expiratory synaptic activity. This intensified differentiation is not restricted to effects arising from stimulation of the superior laryngeal nerve: an effect of the kind shown in Fig. 9*D* may be brought out considerably more prominently in response to appropriate stimulation within the hypothalamus (D. Ballantyne, D. Jordan, M. Wood & K. M. Spyer, unpublished data). The distinction was, of course, most apparent when the effect of superior laryngeal nerve stimulation was to elicit a clear wave of post-inspiratory i.p.s.p.s. Again, the occurrence of these i.p.s.p.s. was not simply a specific response to laryngeal stimulation since they also occurred spontaneously.

There would seem to be no absolute distinction between neurones which receive post-inspiratory i.p.s.p.s and those which do not, since adequate stimulation of what were probably 'irritant' laryngeal receptors evoked a wave of post-inspiratory i.p.s.p.s in 70% of the neurones which did not show them spontaneously. Moreover, in a given neurone there was a wide-ranging variation in the shape of the expiratory phase membrane potential pattern with changes in the pattern of respiration, a feature which suggests that the sample was fairly homogeneous. It is not immediately obvious, however, why this phasic pattern of inhibition was 'spontaneously' so well developed in some neurones and absent in others. One possibility is that it was due to differences in the state of the preparation, including anaesthetic level (increasing which exerted a severely depressive effect on spontaneous post-inspiratory i.p.s.p.s in Fig. 5) and perhaps the prevailing state of chemical respiratory 'drive'.

Although the function of post-inspiratory inhibition would seem to be one of modulating the structure of the expiratory 'burst' (time of onset, rate at which discharge frequency rises), it might be utilized in a number of different circumstances, i.e. it may be activated by a variety of afferent inputs, possibly including peripheral chemoreceptors (see fig. 5*C* of Lipski, Trzebski, Chodobska & Kruk, 1984) as well as laryngeal receptors. If this is the case, the relationship of post-inspiratory inhibition to other aspects of respiratory activity might very well vary, depending on the way each of these inputs is distributed to medullary respiratory neurones. An explanation of this kind might ultimately account for the variable relationship between these i.p.s.p.s and post-inspiratory phrenic activity.

Mechanisms modulating expiratory synaptic activity

The most elementary requirements for rhythmic expiratory activity may be satisfied by a periodic (inspiratory-related) inhibition of neurones subject to a tonic pattern of excitation (Bainton *et al.* 1978; Bainton & Kirkwood, 1979). The nearest approach to a pattern of this kind observed here was that shown in Fig. 5*D*. This pattern is, however, modifiable by inhibitory processes operating in, and defining, the post-inspiratory component of the expiratory interval. We would suggest that post-inspiratory i.p.s.p.s are generated by some (unknown) fraction of the population of medullary 'post-inspiratory' neurones which exhibit both the requisite discharge pattern (Richter, 1982), and which in the present experiments were occasionally encountered as isolated individuals within the same electrode track as 'late expiratory' neurones. Moreover, these neurones are excited at short latency by stimulation of the superior laryngeal nerve (Remmers *et al.* 1985), while 'late expiratory' neurones are inhibited (Ballantyne & Richter, 1982*a*) i.e. their short latency reflex response is antagonistic, as is their longer term response to this kind of stimulation.

It is not entirely clear whether the synaptic control of the post-inspiratory component of the membrane potential trajectory is exerted primarily through the interaction of i.p.s.p.s with an ongoing e.p.s.p. pattern or, as mentioned in connexion with Fig. 9, whether the e.p.s.p. input may also be 'gated out' at some antecedent level, i.e. by a mechanism not necessarily involving post-synaptic inhibition at bulbospinal neurones. Presynaptic inhibition of the excitatory input to bulbospinal neurones is an obvious possibility in this respect, and it might also be achieved by the same inhibitory neurones (or some fraction of the population) responsible for post-synaptic inhibition during this part of the respiratory cycle. Inasmuch as the anticipated effect of both kinds of inhibitory process would be to slow the rate at which threshold for discharge is approached, they might be regarded as two components of a common mechanism designed to 'time' the onset of the expiratory burst. The results do not enable a firm decision to be reached on this point but since the neurones tested in this sample divided in a fairly straightforward way between those which behaved like that of Fig. 4 and those which behaved like that of Fig. 9 (the majority), our inclination is to favour the notion that both post-synaptic inhibition and disfacilitation (by whatever mechanism) may be involved in early expiration.

Quite apart from the action of inhibitory processes operating fairly directly at bulbospinal level, the possibility also exists that the low level of post-inspiratory synaptic noise shown in Fig. 9*B–D* is not a 'phasic' effect related specifically to expiration but is part of a more general disfacilitation of expiratory bulbospinal neurones which coincides with inspiratory inhibition. Its *effect* (i.e. as a phase of reduced synaptic noise), may be detectable only when inhibition is lifted.

Finally, the possibility also arises that inhibitory mechanisms may exert a more general role in shaping the expiratory phase membrane potential trajectory in addition to the phasic pattern of post-inspiratory inhibition. Although no evidence for other patterns of inhibition was obtained here, it is of considerable interest that Bötzinger expiratory neurones, which are inhibitory at all their known output connexions within the medullar and spinal cord, also project to the caudal expiratory

region of the medulla (Merrill, Lipski, Kubin & Fedorko, 1983; Fedorko & Merrill, 1984). Their discharge pattern, essentially the same as that of caudal 'late expiratory' neurones, would seem to preclude their having a role in the post-inspiratory inhibition of the latter.

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