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# DISCHARGE PATTERNS OF CERVICAL SYMPATHETIC PREGANGLIONIC NEURONES RELATED TO CENTRAL RESPIRATORY DRIVE IN THE RAT

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## SUMMARY

1. The central respiratory-drive-related inputs to antidromically identified cervical sympathetic preganglionic neurones have been investigated, in the rat, using extracellular recording techniques, the ionophoretic application of an excitatory amino acid (glutamate) to increase their excitability, and phrenic nerve discharge as an indicator of central respiratory drive.

2. Three distinct firing patterns of sympathetic preganglionic neurones are described: maximal discharge during phrenic nerve activity, maximal discharge during phrenic silence, and a firing pattern unrelated to phrenic nerve discharge.

3. Both spontaneously active and glutamate-activated silent cervical sympathetic preganglionic neurones had similar, if not identical, firing patterns.

4. The application of glutamate, using ionophoretic currents of up to 100 nA, did not disrupt central respiratory-drive-related discharge patterns indicating that these inputs are an important contribution in the regulation of the firing pattern of a proportion of sympathetic preganglionic neurones.

5. On the basis of these observations it is proposed that some sympathetic preganglionic neurones may receive central respiratory drive potentials similar to those received by respiratory motoneurones.

## INTRODUCTION

A respiratory-related discharge pattern of sympathetic nerve activity was first observed by Adrian, Bronk & Phillips (1932) in cervical and abdominal sympathetic nerve recordings in cat and rabbit. Since these initial observations a respiratory grouping of activity has been observed in many sympathetic nerves, although the exact nature of these periodicities depends on the type of preparation (cf. Barman & Gebber, 1976), inspiratory and expiratory peaks are often observed in whole sympathetic nerve recordings (e.g. Cohen & Gootman, 1970; Bainton, Richter, Seller, Ballantyne & Klein, 1985). However, as sympathetic nerves do not consist of functionally homogenous populations of neurones (Preiss, Kirchner & Polosa, 1975; Janig & Szulczyk, 1980), single neuronal activity must be studied in order to: (i) gain

detailed information regarding the relationship of sympathetic neurone discharge to central respiratory drive and (ii) determine the mechanisms responsible for generating the patterns of discharge.

Polosa and his colleagues (Preiss *et al.* 1975; Preiss & Polosa, 1977; Gerber & Polosa, 1978, 1979) have studied the respiratory modulation of cervical sympathetic preganglionic neurones in the cat by analysing the discharge patterns of single fibres using phrenic nerve discharge as an indicator of central respiratory drive. They reported four major patterns of discharge: inspiratory burst, continuous with inspiratory peak, expiratory and non-modulated. However, fibre recordings do not allow any assessment of the subthreshold influences of respiration on sympathetic activity. There are indications that such inputs may be substantial as Lipski, Coote & Trzebski (1977) observed shifts in antidromic latencies suggesting that some silent neurones may receive synaptic inputs related to central respiratory drive. The importance of studying the central respiratory-drive-related inputs to silent neurones is emphasized by the observation that as many as 75 % of sympathetic preganglionic neurones may be silent in the anaesthetized preparation (Gilbey, Coote & Peterson, 1982*b*).

The present investigation has been directed at the question as to whether silent neurones receive central respiratory-drive-related inputs similar to those received by spontaneously active neurones. Recordings have been made from the cell bodies of cervical sympathetic preganglionic neurones enabling them to be excited by the ionophoresis of glutamate. In this way it has proved possible to determine whether silent neurones receive subthreshold respiratory-related inputs of similar form to those received by spontaneously active neurones.

Some of the present results have appeared in abstract form (Gilbey, Jordan, Numao, Spyer & Wood, 1985).

## METHODS

Experiments were carried out on Sprague–Dawley rats (250–350 g) which were anaesthetized with an I.P. injection of sodium pentobarbitone (Sagatal, May & Baker, 50 mg/kg). Supplementary doses of anaesthetic were given when required, as judged from recordings of blood pressure, heart rate and phrenic nerve activity, in the form of I.V. chloralose (5–10 mg). In all cases the trachea was cannulated low in the neck, and catheters placed in a femoral artery and vein for monitoring arterial blood pressure and the administration of drugs, respectively. Animals were artificially ventilated on oxygen-enriched room air following paralysis with gallamine triethiodide (8 mg/kg). Blood gases were monitored (Corning 158 pH/Blood Gas Analyzer) and partial pressure of arterial $CO_2$ ($P_{a,CO_2}$) maintained between 35 and 48 torr and partial pressure of arterial $O_2$ ($P_{a,O_2}$) between 200 and 300 torr in control conditions. Blood samples were taken from a femoral artery (0·5 ml) and the volume removed immediately replaced by donor blood. End-tidal $CO_2$ was sampled intermittently (P. K. Morgan Ltd., 901 Mk 2, $CO_2$ analyzer). Most of the neurones (fifty-five of eighty-one) were studied in animals which were vagotomized and given a pneumothorax as it was noted in the earlier experiments that although the animals were artificially ventilated at rates (60–120/min) several times higher than the frequency of phrenic bursts (typically about 3 : 1), in order to dissociate the lung inflation cycle from central respiratory drive, the intrinsic respiratory rhythm often became phase locked to the pump rhythm. When animals were given a pneumothorax, an end expiratory pressure of 2–3 cm$H_2O$ was applied to the expiratory line to prevent atelectasis. Rectal temperature was maintained between 37 °C ± 1 °C with a heating blanket controlled by a feed-back circuit.

Following a mid-dorsal incision and retraction of the scapula the left cervical sympathetic and phrenic nerves were isolated. The phrenic nerve was cut and activity recorded from the cut and

desheathed central end. A laminectomy was performed to expose the Th2 segment. Sympathetic preganglionic neurones projecting to the cervical sympathetic nerve were antidromically identified, and spinal cord stabilized as described previously (Gilbey *et al.* 1982*b*). Five-barrelled micropipettes were used for recording extracellular activity from sympathetic preganglionic neurones and for the ionophoretic application of glutamate (for details see Gilbey, Coote, Fleetwood-Walker & Peterson, 1982*a*). The recording barrel contained 4 M-NaCl; other barrels contained 0·2 M-L-glutamic acid, 1 M-NaCl for current balancing and either glycine (0·5 M, pH 3·5) or $\gamma$-aminobutyric acid (GABA, 1 M, pH 4·2). Inhibitory amino acids were used to reduce the firing rates of sympathetic preganglionic neurones so that cancellation could be observed (see later).

*Data collection*

Sympathetic preganglionic neuronal activity, phrenic nerve activity, blood pressure, tracheal pressure and electro-cardiogram were stored on tape (Racal 7DS) for off-line analysis.

*Data analysis*

To generate phrenic-triggered histograms phrenic nerve activity was fed into an integrator (Neurolog, NL 703). This output was led into an interface (Neurolog, NL 515) which generated a TTL pulse when integrated phrenic nerve activity reached a preset level. The TTL pulse so generated was used to trigger a minicomputer (Cambridge Electronic Design, Slam System). Sympathetic preganglionic neuronal activity was fed into a spike processor (Digitimer D130) which generated TTL pulses which were delivered to the computer. Thus, phrenic-triggered histograms were generated. Peri-phrenic histograms were constructed by passing the spike processor output into a pulse shift module (Neurolog, NL 730). Integrated phrenic nerve activity was averaged using the minicomputer. An analog delay (Neurolog, NL 740) was introduced into the line to construct the peri-phrenic average.

## RESULTS

Sympathetic preganglionic neurones projecting to the cervical sympathetic nerve were identified antidromically using standard criteria as described previously (Gilbey *et al.* 1982*b*). Briefly these were a sharp threshold for activation, a constant latency of the response and most importantly the cancellation of a spontaneous or glutamate-evoked action potential with the antidromically evoked action potential (Fig. 1*A*). When the interspike interval was shorter than the critical period (range of critical periods 22–64 ms) glycine or GABA were ionophoretically applied to reduce the firing rate so that cancellation could be observed. Estimated axonal conduction velocities were similar to those previously reported (Gilbey *et al.* 1982*b*) and are shown in Fig. 1*B*.

Eighty-one sympathetic preganglionic neurones were studied. Fifty-nine had their activity analysed for a phrenic-related discharge pattern, of these thirty-eight were spontaneously active and twenty-one were silent but could be caused to discharge by the ionophoretic application of glutamate. The remaining neurones (twenty-two) could not be activated with glutamate and in consequence were not studied further.

Three distinct patterns of discharge are reported in this paper, corresponding to neurones firing maximally during the period of phrenic nerve discharge, those discharging maximally during phrenic silence and those which had a firing pattern with no clear relation to phrenic nerve discharge. The initial activity pattern recorded from an individual neurone was maintained throughout the period of recording when $P_{a,CO_2}$ was maintained within control values. Such observations were made for periods of up to $1\frac{1}{2}$ h. Neurones exhibiting these distinct patterns of discharge will be referred to as inspiratory-related, expiratory-related and non-modulated respectively.

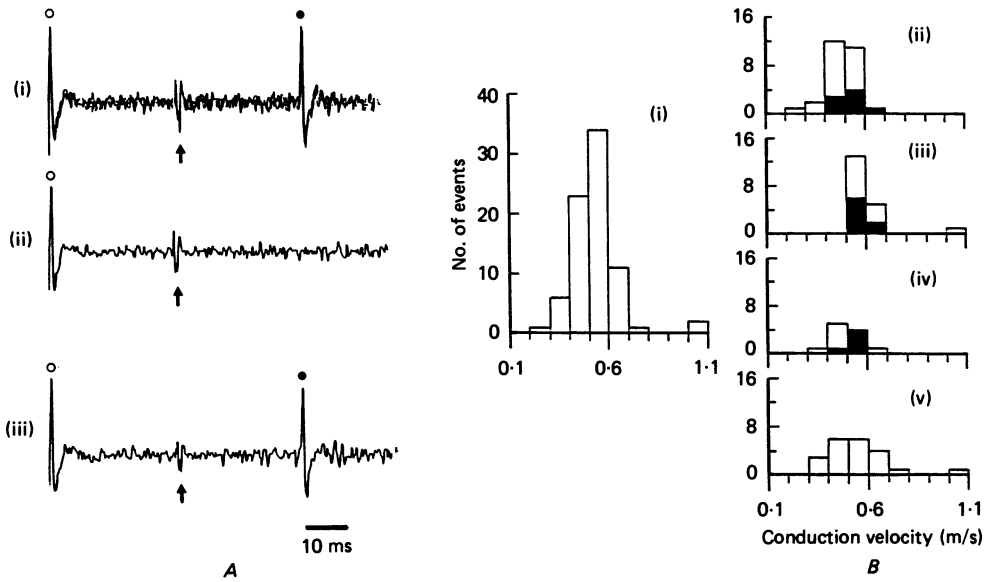


Fig. 1. *A*, antidromic identification of a cervical sympathetic preganglionic neurone by collision testing using a spontaneous action potential to trigger the antidromic stimulus. (i), shows five superimposed antidromic action potentials; (ii), the delay between the spontaneous spike and the stimulus to the cervical sympathetic nerve has been reduced to 32 ms and collision has occurred; (iii), the antidromic action potential reappears when delay is increased to 33 ms. Open circles indicate spontaneous spike; filled circles, antidromic spike; arrows indicate stimulus artifacts. *B*, histograms of the calculated conduction velocities of the total population of cervical sympathetic preganglionic neurones (i) and those defined on the basis of firing pattern as expiratory related (ii), inspiratory related (iii), non-modulated (iv) and non-glutamate activated (v). Filled part of histograms represent glutamate-activated neurones.

*Inspiratory-related sympathetic preganglionic neurones*

This population comprised eighteen of fifty-nine cells studied (twelve spontaneously active; six glutamate activated). These neurones were categorized into two types to aid the description of their firing patterns: type 'A' fired with a burst coincident with phrenic nerve discharge and were silent throughout the rest of the respiratory cycle (Fig. 2*A*, *C*); type 'B' fired consistently in phase with phrenic nerve discharge but in addition discharged during late expiration and had a low probability of discharge during early expiration (Fig. 2*B*, *D*).

Seven spontaneously active neurones fired with a type 'A' discharge pattern, the remainder firing with a type 'B' discharge. Of the neurones showing a type 'A' discharge three maintained their firing pattern during the application of glutamate although their discharge was increased with respect to both frequency and duration. Only one neurone had its type 'A' discharge converted to a type 'B' discharge by the ionophoretic application of glutamate (Fig. 3*A*).

Glutamate-activated neurones, six in total, had similar firing patterns to those described for spontaneously active neurones. Three at the lower currents of glutamate fired with a type 'A' discharge which at higher currents was converted to a type 'B' discharge (Fig. 3*B*). One glutamate-activated neurone only fired with a type 'B'

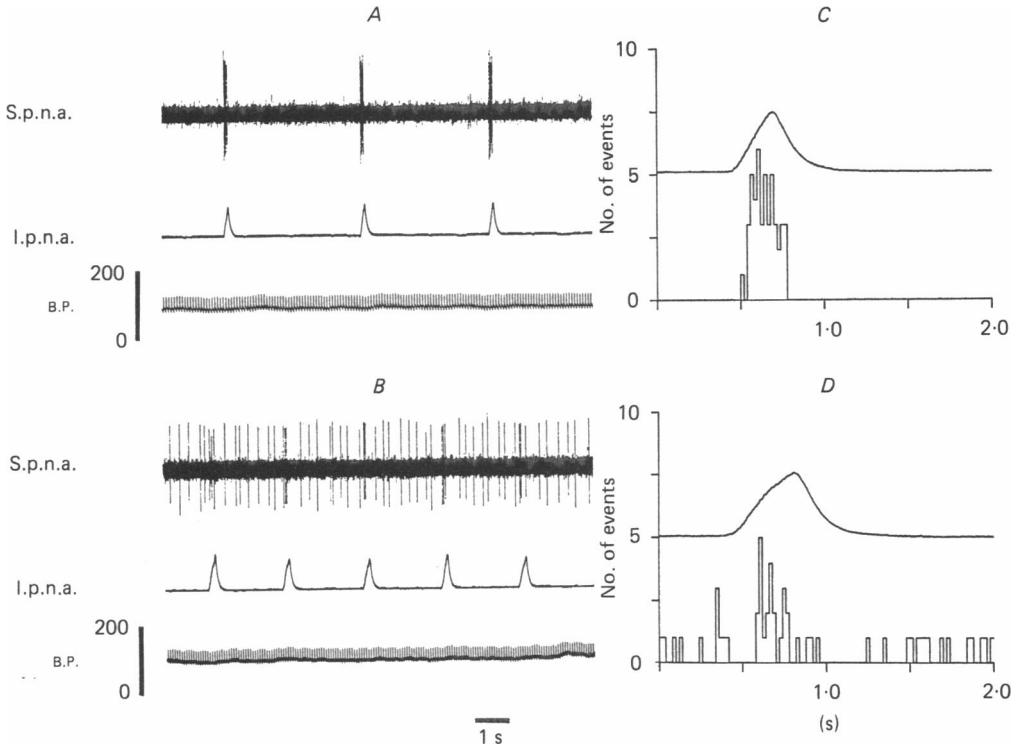


Fig. 2. *A* and *B*, recordings from two different inspiratory-related sympathetic preganglionic neurones showing type 'A' and type 'B' firing patterns, respectively. Traces from above: sympathetic preganglionic neuronal activity (s.p.n.a.), integrated phrenic nerve activity (i.p.n.a.) and femoral arterial blood pressure (B.P.). *C* and *D* are periphrenic histograms of the discharge of *A* and *B* respectively, accumulated over ten phrenic cycles (20 ms bins). Averaged integrated phrenic nerve activity accumulated over the period is shown above each histogram.

discharge (Fig. 3*C*). Two neurones retained a type 'A' discharge pattern even when glutamate was applied with currents of up to 100 nA (Fig. 4).

All inspiratory-related neurones were most resistant to the excitatory effect of glutamate during early expiration, that is the period which follows the peak of the phrenic ramp. This is illustrated in Fig. 3*C* and can be best demonstrated when the ionophoretic current of glutamate is increased in steps (Fig. 4). As the current of glutamate was increased the phrenic-related discharge began earlier but, in contrast, the timing of the off-switching was relatively unaffected (compare Fig. 4*B*, *C*, *D* and *E*). Another characteristic of the effect of glutamate was that the firing rate could not be increased beyond about 40 Hz. Once this firing rate had been attained the period of firing could still be increased but the maximum firing rate tended to decrease.

When the $P_{a,CO_2}$ was reduced to just below that required for rhythmic phrenic discharge by hyperventilation, type 'A' neurones became silent and type 'B' neurones discharged continuously at a rate similar to that observed during late expiration (Fig. 5*A*, *B*). When central respiratory drive was weak, or irregular, neurones of the 'A' type sometimes failed to fire in a burst coincident with phrenic nerve discharge (Fig. 5*C*, *D*).

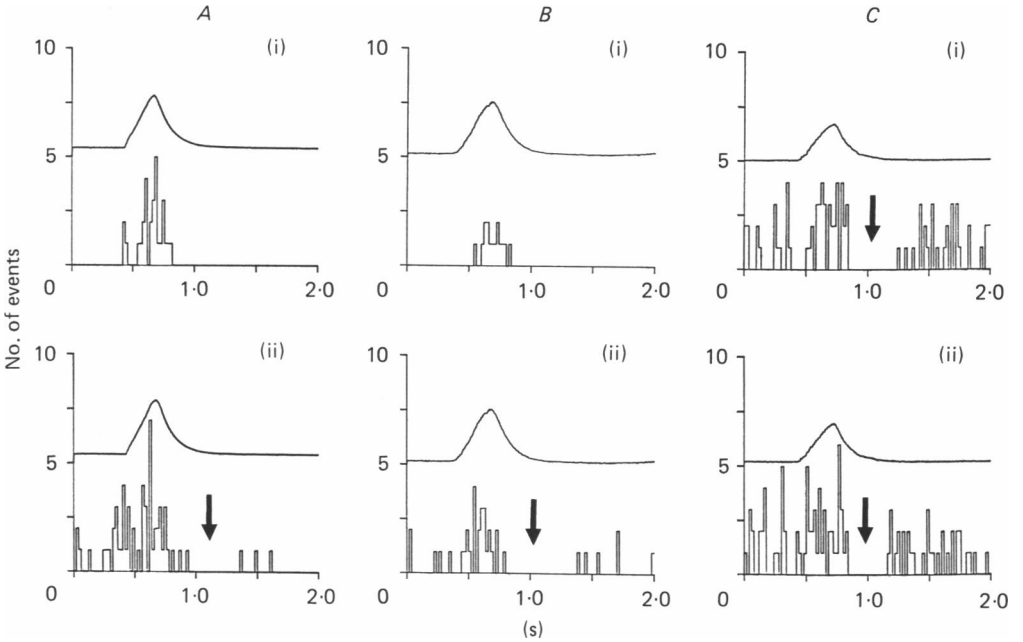


Fig. 3. Periphrenic histograms as shown in Fig.2. *A* shows a type 'A' discharge (i) which was converted to a type 'B' discharge by the ionophoretic application of glutamate (ii). *B*, glutamate-activated silent neurone showing a type 'A' discharge when the retaining current was reduced to +3 nA (i) which was converted to a type 'B' discharge when the ionophoretic current was increased to −10 nA (ii). *C* shows a glutamate-activated silent neurone that only showed a type 'B' discharge when activated with glutamate 0 nA (i) and 10 nA (ii). Arrows indicate period when neurones were most resistant to the excitatory effect of glutamate.

*Expiratory-related sympathetic preganglionic neurones*

Expiratory-related sympathetic preganglionic neurones had relatively uncomplicated patterns of discharge compared to those observed for inspiratory-related sympathetic preganglionic neurones. These fired maximally during phrenic silence and had their lowest probability of firing during phrenic nerve discharge, which can be seen as clearly visible periods of silence in the neurogram (Fig. 6*A*). Twenty-seven neurones displayed this type of discharge pattern of which eight were glutamate-activated and otherwise silent neurones.

Both spontaneously active and glutamate-activated neurones had similar, if not identical, firing patterns (Fig. 6*B*, *C*). The neurones could not be made to fire at a constant rate throughout the respiratory cycle by increasing the ionophoretic application of glutamate up to 100 nA. When the phrenic nerve discharge was silenced by hyperventilation these neurones fired continuously (Fig. 7).

*Non-modulated sympathetic preganglionic neurones*

These neurones had an activity pattern which was unrelated to phrenic nerve discharge at $P_{a,CO_2}$ levels up to 60 torr when central respiratory drive was always very marked. This group included six spontaneously active neurones and five glutamate-activated neurones.

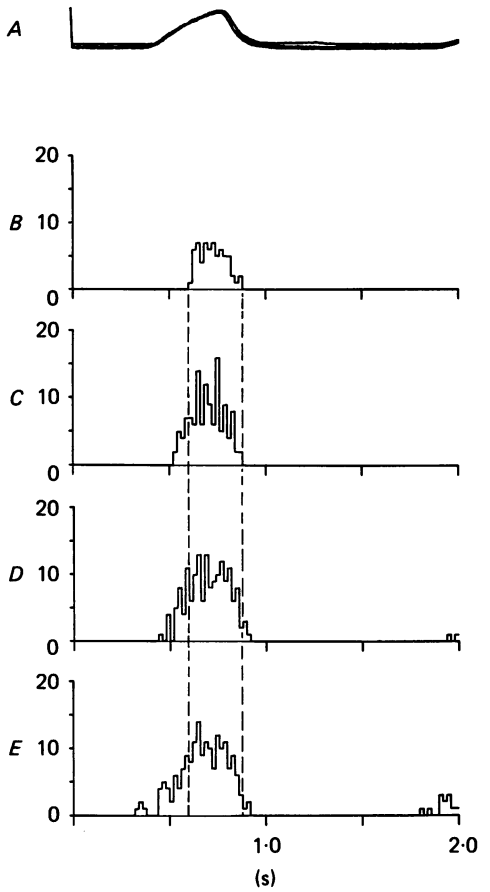


Fig. 4. Periphrenic histograms; *B*, *C*, *D* and *E* show the effect of the ionophoretic application of glutamate, using currents of 10, 30, 50 and 100 nA respectively, on the type 'A' discharge of a glutamate-activated, and otherwise, silent neurone (twenty sweeps, 20 ms bins). *A*, shows the average superimposed phrenic activity during the periods when *B*, *C*, *D* and *E* were accumulated. Observe that the timing of the off-switching changes little compared with the advance of onset of the inspiratory synchronous discharge.

## DISCUSSION

The data presented in this study show that in the rat many neurones projecting to the cervical sympathetic nerve have distinct phrenic-related discharge patterns similar to those which have been observed in cervical sympathetic preganglionic neurones in the cat (Preiss *et al.* 1975). These patterns of discharge were present in animals which had a pneumothorax and were vagotomized, and also in those which had intact vagi. In all animals artificial ventilation was effected at rates several times higher than the frequency of phrenic bursts, therefore dissociating the lung inflation cycle from central respiratory drive. Thus these patterns are by inference related to central respiratory drive and cannot result from afferent feed-back related to lung inflation. Furthermore, as there was an absence of blood pressure waves in parallel with central respiratory activity, variable arterial baroreceptor input is unlikely to have contributed to the respiratory-related discharges.

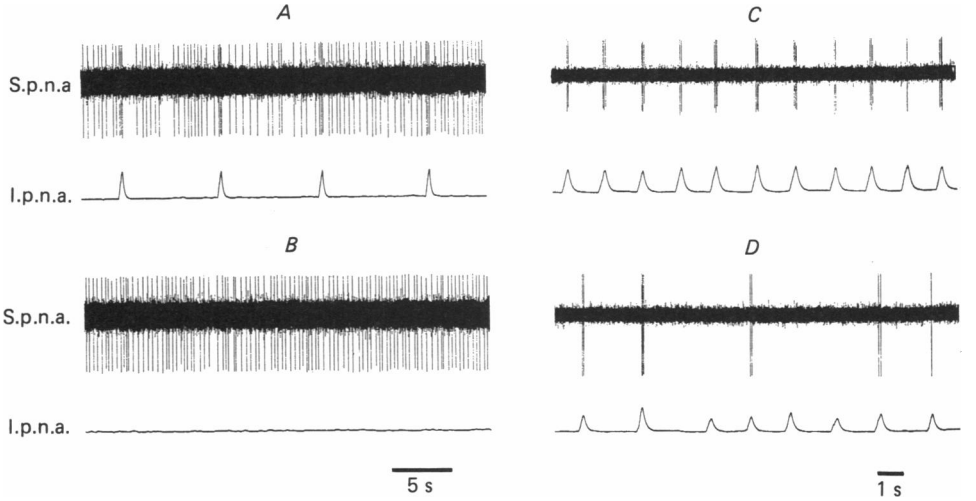


Fig. 5. *A* shows the type 'B' discharge of a unit in control period and *B*, the same neurone discharging when the phrenic activity had been silenced by hyperventilation. *C* shows a unit with a type 'A' discharge in the control situation which when ventilation was increased (*D*), producing an irregular phrenic nerve discharge, the neurone did not discharge during every respiratory cycle. S.p.n.a., sympathetic preganglionic neuronal activity; i.p.n.a., integrated phrenic nerve activity.

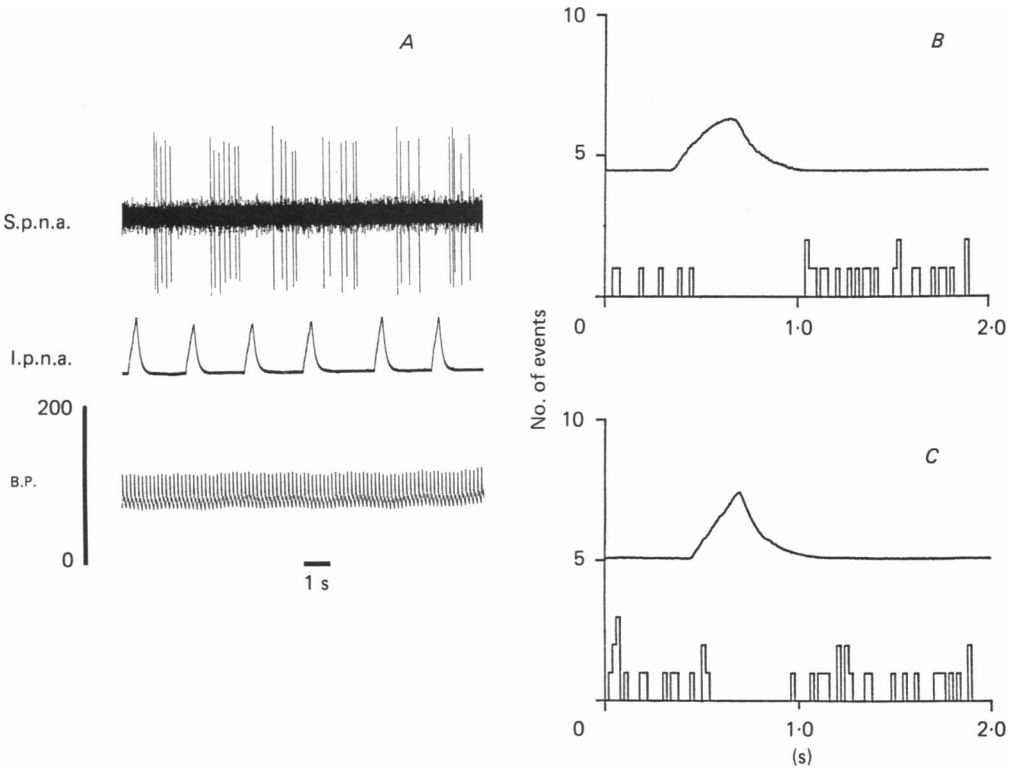


Fig. 6. *A* is a recording from a spontaneously active expiratory-related preganglionic neurone. Traces from above sympathetic preganglionic neuronal activity (s.p.n.a.), integrated phrenic nerve activity (i.p.n.a.) and femoral arterial blood pressure (B.P.). Periphrenic histograms (accumulated over ten respiratory cycles): *B*, activity of neurone shown in *A*; *C*, glutamate-activated neurone showing a similar rhythm.

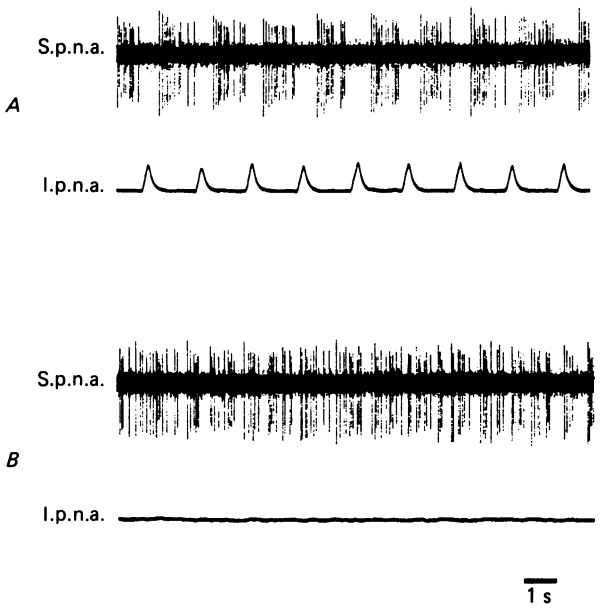


Fig. 7. *A* shows neurone with expiratory discharge pattern which continues to fire when the phrenic nerve discharge is silenced during hyperventilation (*B*). S.p.n.a., sympathetic preganglionic neurone activity; i.p.n.a., integrated phrenic nerve activity.

In the present study by using the technique of ionophoresis it was possible to observe the discharge patterns of glutamate-activated neurones, in addition to the discharge patterns of spontaneously active neurones. It was observed that some glutamate-activated neurones fired with respiratory-related discharge patterns, which were similar, if not identical, to those observed for spontaneously active neurones. If it is assumed that glutamate acts directly on the sympathetic preganglionic neurone under investigation, rather than on antecedent neurones, these observations provide evidence that some silent neurones receive subthreshold inputs related to central respiratory drive, either directly via bulbospinal neurones or via spinal interneurones, which are substantial enough to determine the spike output when a neurone is brought to threshold by glutamate, or presumably, other powerful excitatory inputs. Thus we have both confirmed and extended the observations of Lipski *et al.* (1977) who measured variations in antidromic latencies of sympathetic preganglionic neurones in the cat, related to central respiratory activity, and proposed that these reflected changes in soma excitability. The importance of the respiratory-related inputs in determining the pattern of spike output of sympathetic preganglionic neurones is emphasized by the fact that ionophoresis of glutamate onto respiratory modulated neurones was never observed to disrupt the respiratory-related discharge patterns, rather acting to modify the frequency of discharge.

*Inspiratory-related sympathetic preganglionic neurones*

Inspiratory-related sympathetic preganglionic neurones were found to be most accessible to the excitatory influences of glutamate during a period coincident with the phrenic ramp. Silent neurones caused to discharge with phrenic-related bursts,

and spontaneously active neurones discharging in a similar manner, had their phrenic-related discharge altered in a stereotyped manner to step increases in the ionophoretic current of glutamate; the onset of the inspiratory synchronous spike discharge began progressively earlier in relation to the beginning of the phrenic discharge and there was an increase in the intra-burst spike frequency until a plateau was reached. However, the timing of the off-switching was relatively unaffected. This can be taken to indicate that inspiratory-related excitatory drive potentials are responsible for the pattern of discharge. It is thus probable that drive potentials onto these neurones are similar to those received by inspiratory motoneurones (Sears, 1964; Berger, 1979). This would explain the action of glutamate in causing some silent neurones to discharge with an inspiratory firing pattern once their general level of excitability has been increased by glutamate allowing the inspiratory-related excitatory drive potentials to exceed threshold. It would then be predicted that the more a neurone was depolarized by glutamate the earlier the neurone would discharge, and our observations confirm this. With regard to the sudden cessation of the inspiratory burst, this may be brought about by any one of a number of mechanisms: post-excitatory depression (Mannard, Rajchgot & Polosa, 1977) resulting from loading of the cell with sodium during high-frequency firing and subsequent extrusion by an electrogenic sodium–potassium pump (Saum, Brown & Tuley, 1976) or an increase in calcium-activated potassium conductance (Meech, 1978); disfacilitation and/or shunting of excitatory post-synaptic potentials (e.p.s.p.s) by inhibitory post-synaptic potentials (i.p.s.p.s), as observed in inspiratory motoneurones (Sears, 1964; Berger, 1979). Such disfacilitation, or inhibition, could arise from direct or indirect connexions with 'late' inspiratory neurones (Richter & Ballantyne, 1983). Unfortunately, there is no information regarding these possibilities from intracellular data as synaptic inputs to sympathetic preganglionic neurones related to central respiratory drive remain to be investigated (Fernandez de Molina, Kuno & Perl, 1965; Coote & Westbury, 1979; McLachlan & Hirst, 1980; Dembowsky, Czachurski & Seller, 1985). However, our own limited observations in the cat support the contention that these neurones receive drive potentials (H. A. Futuro-Neto, M. P. Gilbey, L. M. Wood & K. M. Spyer, unpublished observations).

The striking similarity between the type 'A' firing pattern and that of some inspiratory medullary neurones (Richter & Ballantyne, 1983; Ballantyne & Richter, 1984) indicates that type 'A' firing may derive from inputs from either inspiratory medullary neurones (direct or via segmental or propriospinal interneurones) or from synaptic inputs shared with inspiratory medullary neurones (at present the latter appears more likely, see Kubin, Trzebski & Lipski, 1985; Connelly & Wurster, 1985). Typically inspiratory medullary neurones receive an increasing number of e.p.s.p.s during inspiration which are shunted by i.p.s.p.s at the end of inspiration; these probably arising from late inspiratory neurones (Richter & Ballantyne, 1983; Ballantyne & Richter, 1984). Similar synaptic mechanisms may explain the refractoriness of inspiratory-related sympathetic preganglionic neurones to the excitatory influences of glutamate during early (Stage I) expiration. However, the behaviour of some inspiratory-related sympathetic preganglionic neurones is not consistent with them receiving a wave of i.p.s.p.s during late (Stage II) expiration as do medullary inspiratory neurones (Ballantyne & Richter, 1984); some fired with a type 'B'

discharge or could have their type 'A' discharge converted to a type 'B' discharge by the ionophoresis of glutamate.

Inspiratory-related preganglionic neurones may receive excitatory drive independent of central respiratory drive since inspiratory-related sympathetic preganglionic neurones with a type 'B' discharge became tonically active when phrenic nerve discharge was silenced. Consequently, inspiratory-related sympathetic preganglionic neurones may receive at least two excitatory drives; one dependent on the respiratory state of the animal and the second derived from other sources. Whether inspiratory-related preganglionic neurones discharge with a type 'A' or type 'B' firing pattern may simply reflect their state of activation as in the case of those illustrated in Fig. 3*A* and *B*. Together with possible inhibitory inputs these two drives may then interact to sculpture the firing pattern of these neurones.

*Expiratory-related sympathetic preganglionic neurones*

As many glutamate-activated neurones fired with an expiratory pattern of discharge which was similar, if not identical, to that observed in some spontaneously active neurones, it appears that the phasic inputs received by these neurones have a marked control over their firing pattern. In agreement with Preiss *et al.* (1975) it was found that neurones showing this type of discharge pattern in the control situation became tonically active when rhythmic phrenic discharge was abolished by hyperventilation. Thus the periodic interruption of the tonic discharge observed in normocapnia which imposes the expiratory rhythm on this set of neurones may be due to phasic inhibition and/or disfacilitation coincident with inspiration as observed in internal intercostal motoneurones, medullary expiratory neurones and vagal cardio-inhibitory motoneurones (Sears, 1964; Jordan & Spyer, 1981; Gilbey, Jordan, Richter & Spyer, 1984; Ballantyne & Richter, 1986). The source of the excitatory drive to expiratory-related sympathetic preganglionic neurones is unknown and requires further investigation, although it does not appear to be strongly $CO_2$ dependent (Preiss & Polosa, 1977) and in this respect appears to differ from the excitatory drive to expiratory intercostal motoneurones and medullary expiratory neurones (Cohen, 1968; Bainton & Kirkwood, 1979).

In the present series of experiments about 50 % of the sympathetic preganglionic neurones studied displayed an expiratory firing pattern. Thus the frequency with which expiratory-related sympathetic preganglionic neurones were encountered was much greater than that in the study of Preiss *et al.* (1975) and Preiss & Polosa (1977) in the cat. The difference may be due to a number of factors: species, anaesthetic or technique. Consequently, the present preparation appears to be ideally suited to the further study of expiratory-related sympathetic preganglionic neurones, the mechanisms governing their discharge and the functional significance of the discharge.

*Functional implications*

The study has demonstrated that in the rat, as in the cat, many cervical sympathetic preganglionic neurones receive inputs related to central respiratory drive. Some silent neurones appear to receive phasic inputs similar to spontaneously active neurones. These may be revealed when glutamate is applied ionophoretically.

Thus some silent neurones may have functions similar to those of spontaneously active neurones and may be active in the conscious animal or provide a pool of neurones which may be recruited under certain circumstances. As glutamate failed to disrupt the basic firing pattern of respiratory modulated units it has been suggested that the synaptic inputs to these neurones must be powerful and may be in the form of central respiratory drive potentials similar to those received by thoracic respiratory motoneurones.

Although it remains to be determined whether sympathetic preganglionic neurones at other thoracic and lumbar levels display similar patterns of respiratory-related discharge there are indications that neurones discharging in this manner are involved in cardiovascular regulation. Bachoo & Polosa (1985) have recently shown that the discharge of inspiratory-related sympathetic preganglionic neurones may be important in determining vasoconstrictor tone and sympathetic drive to the heart. This proposal is supported by the observation that sympathetic activity in inferior cardiac nerve fibres, renal nerve fibres and skeletal muscle vasoconstrictor fibres supplying the hind limb of the cat (Gregor, Janig & Wilprich, 1977; Bainton *et al.* 1985; M. P. Gilbey, A. G. Ramage & L. M. Wood, unpublished observations) displays a strong inspiratory synchronous discharge. Thus the respiratory modulation of sympathetic outflow may provide one mechanism by which cardiovascular and respiratory functions are co-ordinated.

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