AN ANALYSIS OF
THE INHIBITION OF PHRENIC MOTONEURONES WHICH OCCURS ON STIMULATION OF SOME CRANIAL NERVE AFFERENTS

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SUMMARY

1. Inhibition and excitation of spontaneous phrenic nerve discharges in response to stimulation of the sinus, glossopharyngeal, aortic and superior laryngeal (SLN) nerves has been investigated in cats.

2. The inhibition, in response to a single shock, had a latency of 5–10 msec and lasted for 20–40 msec; the response to SLN stimulation was the most prolonged.

3. Excitation of phrenic motoneurones also occurred and was seen either before or after the end of the inhibition of the inspiratory burst and sometimes also during expiration.

4. Intravenous strychnine blocked the inhibition.

5. Intracellular recording from phrenic motoneurones showed that hyperpolarization was evoked by each nerve during the central phrenic depolarizing potential (CPDP) but only rarely in the interval between these potentials.

6. When the CPDP was suppressed, hyperpolarization could sometimes be evoked.

7. There were no changes in amplitude or time course of hyperpolarization during the passage of current through the cell membrane. No change in membrane conductance could be shown by passing current pulses.

8. Raising the pressure in the carotid sinus also depressed phrenic activity and evoked a hyperpolarization whilst the CPDP was suppressed.

9. Inspiratory interneurones in the brain stem were suppressed by nerve stimulation and by a rise in carotid sinus pressure.

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10. Expiratory interneurones in the brain stem were both excited and suppressed by electrical stimuli and unaffected by a change in carotid sinus pressure.

INTRODUCTION

During the course of an investigation of the central connexions of the sinus, glossopharyngeal, aortic and superior laryngeal nerves (Biscoe & Sampson, 1970*a*, *b*) it was observed that electrical stimulation of each of these nerves caused a profound, relatively short latency depression of spontaneous phrenic nerve activity. This paper describes an investigation of the depression and some associated phenomena. A similar depression of phrenic activity has been described by Megirian (1968) in response to superior laryngeal nerve stimulation.

A preliminary account of some of the observations has been published (Sampson, Biscoe & Campion, 1968).

METHODS

Forty-three cats were used. Twenty cats were anaesthetized with sodium pentobarbitone (Diabutal: Diamond Laboratories) 35 mg/kg given intraperitoneally; fifteen were anaesthetized with Dial-urethane (Ciba) 0·6 ml./kg intraperitoneally. In both these groups supplemental doses of sodium pentobarbitone were given intravenously when required. Eight cats were made decerebrate by electro-coagulation at the level of the superior colliculus by the method of Crawford & Curtis (1966).

Two types of experiments were performed. In the first, nerve potentials were recorded from neurones in the medullary reticular formation with 4 M-NaCl-filled glass microelectrodes inserted from the ventral side. Arterial pressure, end-tidal CO_2 and blood gas tensions were also monitored; the cats were paralysed with gallamine triethiodide (5 mg/kg, I.V.) and artificially ventilated. Details of the techniques are to be found in another paper (Biscoe & Sampson, 1970*a*).

In the second group of experiments recordings were made from phrenic motoneurones. The dorsal surface of the cervical spinal cord was exposed by a laminectomy extending between the second and sixth cervical vertebrae. The animal was securely fixed in a steel frame and the skin flaps were sutured to surrounding metal bars to form a pool that was filled with liquid paraffin at 38° C and was maintained at this temperature with radiant heat. Included within the pool were the peripheral nerves (see below). In some experiments the dura mater was pinned back to surrounding muscle. Stability of the cervical spinal cord proved difficult to obtain by the usual methods: clamping vertebrae, pneumothorax with low volume artificial ventilation. Agar was ineffective when poured over the surface of the cord. Very good stability was obtained by infiltrating the sub-dural space around the cord with agar so that the entire cord was encased in it. This was simply done using a syringe and fine polyethylene catheter.

Recordings were made in the third, fourth and fifth cervical segments with microelectrodes filled with 2 M-K_2SO_4 or 1·2 M potassium citrate and having resistances of 4–40 MΩ. The micro-electrodes were connected by a chlorided silver wire to a conventional unity gain cathode follower having an input impedance of $> 10^{14}$ Ω and grid current of 10^{-14} A. The follower amplifier had facilities for capacitance

neutralization and backing off electrode tip and membrane potentials. A bridge circuit of conventional configuration was incorporated in the cathode follower. The series resistors were $10^9\ \Omega$ and $10^7\ \Omega$; the current which flowed was monitored with a second cathode follower as the voltage dropped across the lower resistor, thus 1 nA $\equiv$ 10 mV. Current pulses or positive or negative DC were available. The cat, and frame to which it was clamped, were grounded by a chlorided silver coil embedded in muscle through a calibrator which was triggered from the time base. Calibrating pulses could then be delivered to the preparation at will. Electrode resistance was monitored *in situ*. Intracellular membrane potentials were monitored with a digital voltmeter and could be recorded on film or one channel of a pen recorder.

Phrenic nerve recording. One phrenic nerve was cut and spontaneous activity was recorded from the central end to indicate the phase of the central depolarizing potential of the phrenic motoneurones (CPDP). The contralateral nerve was used when recordings were made in the spinal cord, the ipsilateral nerve during brain stem experiments. The nerve was mounted on bipolar platinum wire electrodes connected to a Tektronix 122 amplifier and the second gun of the oscilloscope. The vertical amplifier of the second gun was connected to a second audio monitor and to a discriminator, which was used to eliminate noise from the nerve signal and give an output signal of standard size at each nerve impulse above the discriminator setting. This output signal was compared with the input nerve signal to confirm which impulses were counted. The standard output pulses were also led to a rate-meter for continuous monitoring of nerve discharges.

Peripheral nerve stimulation. In the experiments on the spinal cord the ipsilateral phrenic nerve was cut and stimulated at a frequency of 5/sec in order to excite phrenic motoneurones anti dromically. In some experiments the dorsal roots of C 3, 4 and 5 were also cut. When recordings were made from the spinal cord, the nerves (sinus, glossopharyngeal, IX, aortic and superior laryngeal, SLN) were exposed from the dorsal side with the head of the cat ventroflexed; when recordings were made from the brain stem the nerves were exposed from the ventral side. The sinus nerve was usually left intact and the stimulating electrode placed around it. In this way the effects of raising the pressure in the carotid sinus, by the methods described in Biscoe & Sampson (1970a) as well as those of stimulating the nerve trunk could be studied.

The temperature of the cats was kept at 38° C by a thermistor-controlled heating blanket.

RESULTS

Except where otherwise stated the stimulus intensity used was that which evoked the most pronounced, that is shortest latency with longest duration, inhibition of phrenic motoneurones. Inhibitory phenomena more prolonged than this were sometimes observed, but these will not be described in detail since they are not the subject of this paper. Excitatory responses were also sometimes obtained with this stimulus intensity. All the figures in this paper are from paralysed, artificially ventilated animals.

Effects of nerve stimulation on phrenic nerve activity

Depression. No differences were observed between anaesthetized and decerebrate cats with respect to the effects of nerve stimulation on phrenic nerve activity. The depression of the spontaneous phrenic discharge was

observed whether or not the animal was paralysed with gallamine tri-ethiodide and artificially ventilated. Examples of depression caused by stimulation of all four ipsilateral nerves are illustrated in Fig. 1, where the signal is the whole nerve discharge during an inspiratory burst. Responses having a similar time course were seen with the contralateral nerves. The depression had a latency of 5–10 msec and lasted for 20–40 msec; the shortest latency and longest lasting depression occurred with SLN. There
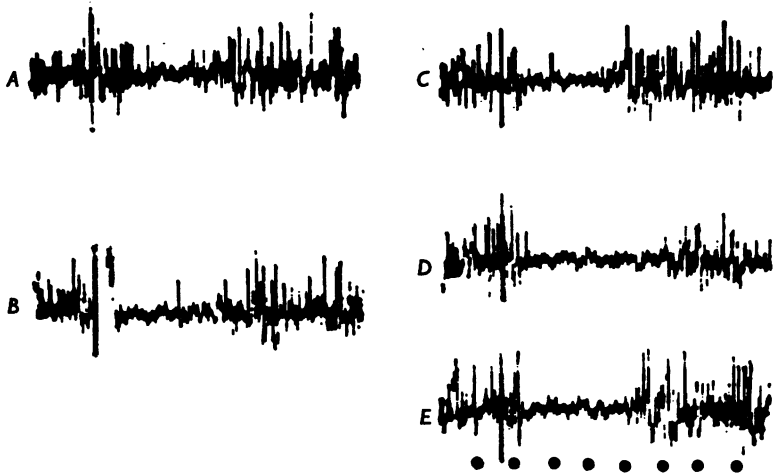

Fig. 1. Cat, Dial-urethane, artificially ventilated. Depression of spontaneous ipsilateral phrenic nerve activity by a single shock to sinus nerve (*A*); aortic nerve (*B*); glossopharyngeal nerve (*C*); superior laryngeal nerve (*D*, *E*). *t* = 10 msec.

was no repeatably observed relationship between the timing of the stimulus during inspiration and the latency or duration of depression, though changes are sometimes seen (Fig. 2*C*, *D*). Depression of phrenic discharges was also demonstrated in few-fibre preparations of the phrenic nerve in response to stimulation of any one of the nerves studied. In Fig. 2*A*, the discharge of the whole nerve is depressed by stimulation of the sinus nerve; in this case the latency was 10 msec and duration 35 msec, though the depression was not complete. The phrenic nerve was then dissected until a strand containing only a few active fibres was found, and five consecutive sweeps of a storage oscilloscope during inspiratory bursts of this strand with no stimulation are shown in Fig. 2*B*. This was repeated, but with a single maximal stimulus to the sinus nerve added and two examples are shown in Fig. 2*C*, *D*. The latency for the depression of these fibres was 10–12 msec, and the duration of depression was 17 msec in *C* and 30 msec in *D* towards the end of the inspiratory burst. There was no evidence for

excitation in this case; the distribution of nerve impulses in *B* shows groups of nerve impulses at frequencies similar to those in *C*, *D*.

The effect of strychnine hydrochloride on the depression of phrenic discharge caused by stimulation of all the nerves was examined. Strychnine 0·1–0·2 mg/kg I.V. reduced the short latency depression after 4 min and abolished it after 6 min. Results from one experiment are illustrated in Fig. 3, where the nerve was stimulated at 1/sec and every response was
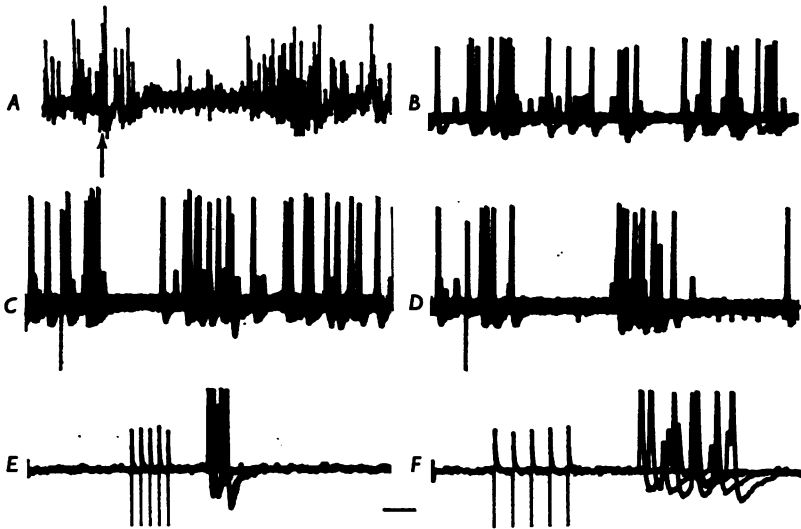

Fig. 2. Cat, pentobarbitone, artificial ventilation. Depression of spontaneous ipsilateral phrenic nerve activity by a single shock to sinus nerve. *A*, whole phrenic nerve, stimulus delivered at the arrow; *B*, few fibre strand from the same phrenic nerve, no stimulus, five sweeps superimposed during the inspiratory discharge; *C*, *D* same as *B* but with a single shock to the sinus nerve; *C* at the beginning of inspiratory burst, *D* at the end of inspiratory burst; *E*, *F*, three sweeps superimposed during expiration to show action potentials evoked by five shocks at 400/sec, same strand as *B* to *D*. *t* = 10 msec in *A* to *E*, = 5 msec in *F*.

photographed. Thus a complete sequence was obtained showing the responses to strychnine. The records show the control effects of IX (*B*) and sinus nerve (*C*) stimulation and thereafter for convenience, the abolition of the effect of sinus nerve stimulation only (*D* to *G*). In Fig. 3*H*, tetanic stimuli (three shocks at 400/sec) partially overcame the strychnine block. The first spontaneous spindles appeared in the phrenic discharge after a total dose of strychnine of 0·8 mg/kg I.V., I; subsequently the spindles could be evoked by a tetanus during expiration, *K*, and later became almost continuous. Similar effects of strychnine on the short latency inhibition were seen with all four nerves.

Excitation. A single stimulus applied to any one of the nerves during expiration usually did not evoke a response in the phrenic nerve. However, a brief tetanus sometimes evoked a burst of action potentials (Fig. 2*E*, *F*), in this cat lasting for 7–15 msec after a latency of 10–12 msec.
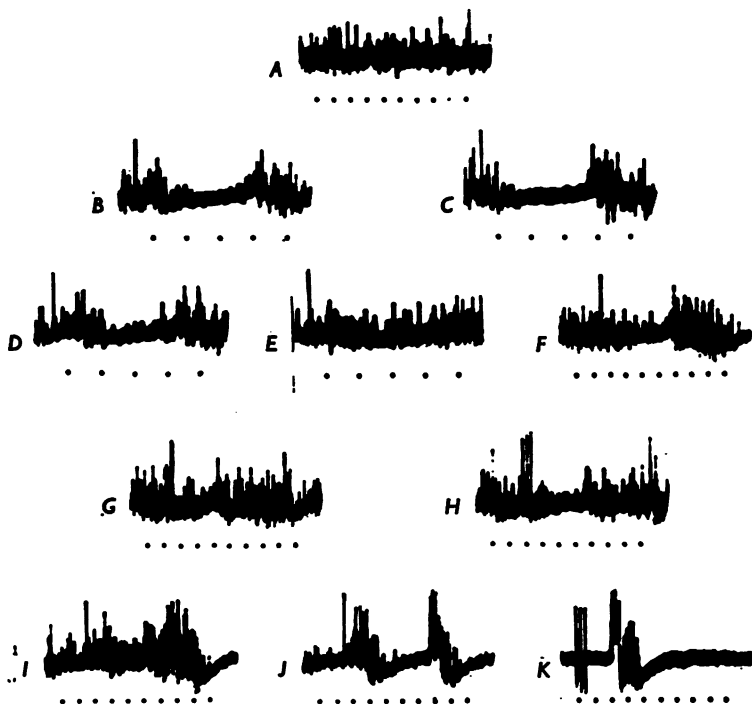

Fig. 3. Cat, Dial-urethane, artificial ventilation. The effect of the administration of strychnine hydrochloride intravenously on the depression of ipsilateral phrenic inspiratory activity by a single shock to the sinus (SN), or the glossopharyngeal (IX), nerves. *A*, no stimulus; *B*, IX stimulus; *C* to *K*, SN stimulus; *D*, 4½ min after strychnine HCl 0·1 mg/kg; *E*, 1½ min after second dose strychnine HCl 0·1 mg/kg, given 5½ min after first dose; *F*, 1½ min after third dose strychnine HCl 0·1 mg/kg, given 11½ min after first dose; *G*, 40 sec after fourth dose strychnine HCl, 0·1 mg/kg, 13½ min after first dose; *H*, stimulus of three shocks at 400/sec, 14 min after first dose; *I*, first spindle activity, 16½ min after first dose; *J*, as *I*; *K*, stimulus three shocks at 400/sec 1½ min after a total dose of 1·1 mg/kg had been given and 25 min after the first dose. *t* = 10 msec.

The excitatory changes which occurred during inspiration were evoked by higher stimulus intensities than those necessary to cause the early inhibition discussed here. The excitation was evident in nerve recordings towards the end of inhibition (Fig. 2*A*). In some experiments the excitation was of sufficient magnitude to obscure the inhibition; compare the

phrenic nerve traces of Fig. 10 (*A, H* with *B, C, I, J*). The accompanying intracellular records of Fig. 10 show that the early hyperpolarization of Fig. 10*A, H* was replaced by a later depolarization when the stimulus intensity was raised, Fig. 10*B, C, I, J*. This depolarization is followed by hyperpolarization, Fig. 10*B, C, E–G, I, J, L–N*, associated with suppression of the phrenic nerve discharge, Fig. 10*B, C, I, J*. Repetitive stimulation is associated with more complex effects of both an inhibitory and excitatory nature with the introduction of an inhibitory effect having a much longer time scale. These excitatory, and long duration inhibitory, phenomena are not the subject of this investigation.

The effects of raising the pressure in the carotid sinus on phrenic nerve activity

This effect was studied qualitatively and brief pressure pulses were applied to the carotid sinus. (The wave form of the pulses is partially indicated in Figs. 6 and 11; the pressure rose to approximately 250 mm Hg for a period between 200 and 600 msec and then fell to the control level asymptotically.) Pressure pulses of this type were invariably associated with depression of the phrenic discharge. Examples of the responses are shown in Fig. 4, where *A* is a control phrenic nerve burst recorded from the whole nerve and *B* shows the depression of the discharge by a pressure pulse. The discharges recorded in a dissected strand of the phrenic nerve were also depressed when the pressure in the carotid sinus was raised (Fig. 4*C*). In other experiments the sinus nerve discharge was monitored in parallel with that from the phrenic nerve so that an indication was given of the baroreceptor activity, a crude indication because this was obtained from the whole sinus nerve. The results from such an experiment are shown in Fig. 4*D, E*; the upper trace is the sinus nerve activity. Each time the pressure was raised bursts of baroreceptor discharges occurred and the activity of the phrenic unit was depressed.

The nature of the short latency effects

Clearly the strychnine sensitive short-latency inhibition of spontaneous phrenic activity may have two separate causes. It may arise from a depression of the phrenic motoneurone central depolarizing potential (CPDP) secondary to either depression of reticular inspiratory units, or excitation of reticular expiratory units, or it may arise from both causes acting in concert. The inhibition may also, or perhaps uniquely, arise from hyperpolarization of phrenic motoneurones by a direct pathway from the brain stem. The former possibility is difficult to analyse until a better understanding is available of the interrelationships of these reticular neurones. However, we have made a number of observations on both 'inspiratory'

and 'expiratory' neurones in an attempt to clarify the nature of the inhibition and an account of effects of nerve stimulation on other cells is given elsewhere (Biscoe & Sampson, 1970*b*). The observations on the phrenic motoneurones follow those on reticular units.

Changes in reticular formation unit activity

Cells which discharge in phase with the phrenic inspiratory burst. Cells that discharged rhythmically in phase with the phrenic burst were depressed by all the stimuli that depressed the phrenic discharge. Fig. 5 shows an example of the depression of an inspiratory unit following electrical
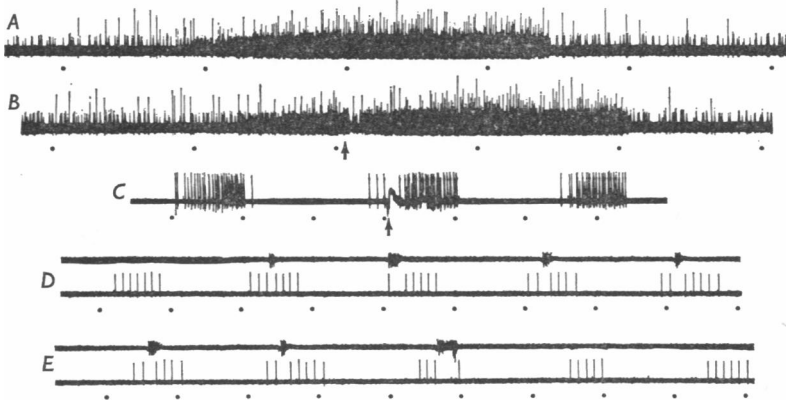

Fig. 4. *A* to *C*, *D* to *E*, from two different cats, both anaesthetized with pentobarbitone and artificially ventilated. *A*, whole nerve inspiratory burst; *B*, as *A* with a rise in carotid sinus pressure at the arrow; *C*, a strand dissected from this nerve showing depression of activity by a rise in pressure in the carotid sinus at the arrow. *D*, *E*, upper trace whole intact sinus nerve recording, lower trace single phrenic fibre. The sinus nerve showed bursts of activity when the pressure in the sinus was raised. Note the depression of phrenic activity synchronous with this. *t* = 1 sec.

stimulation of each of the four nerves; it is difficult to time the latency of this depression accurately because the cells have a low frequency of spontaneous discharge, but the depression lasted for about 30 msec in each case. The record of the whole phrenic nerve also shows a parallel depression. The discharge of other inspiratory units was found to be depressed by a rise in carotid sinus pressure. Fig. 6 illustrates this phenomenon. The control inspiratory bursts for this neurone lasted for approximately 1 sec, whereas that recorded while pressure in the sinus was raised lasted for only 850 msec.

Cells which discharge out of phase with the phrenic inspiratory burst

There were varied effects of nerve stimulation on the discharge of these cells. The activity of one type of unit which discharged during expiration
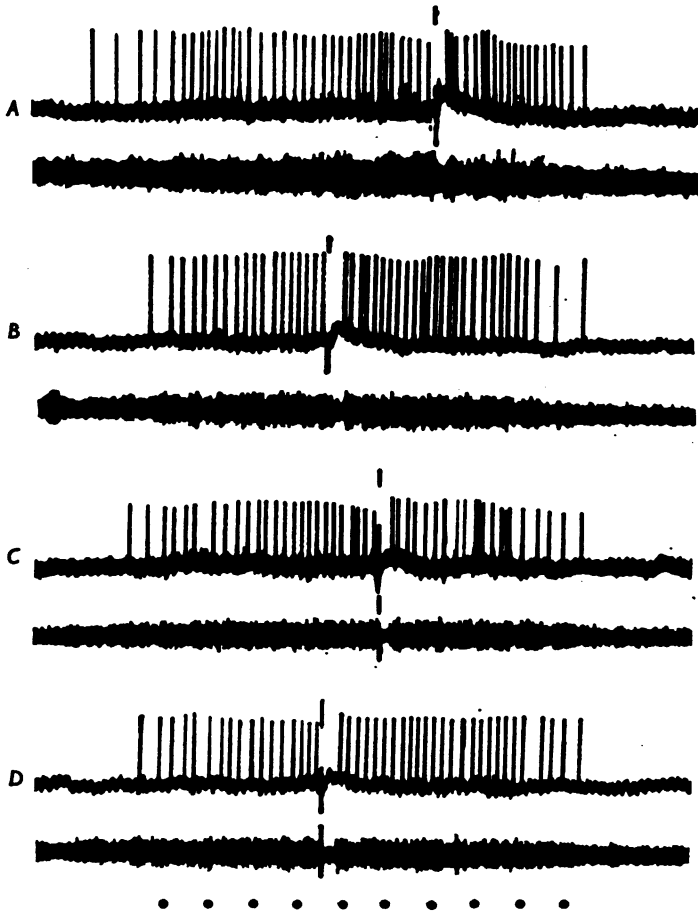
Fig. 5. Cat, Dial-urethane, artificial ventilation. In each case upper trace is micro-electrode recording of an inspiratory unit discharge in the reticular formation (co-ordinates, A.P. − 13, lateral 2·6, depth 4·20 mm), lower trace is ipsilateral phrenic discharge. Single shocks were delivered to *A*, sinus nerve; *B*, glossopharyngeal nerve; *C*, aortic nerve; *D*, superior laryngeal. *t* = 100 msec.

Fig. 6. Cat, Dial-urethane, artificial ventilation. Upper trace micro-electrode recording inspiratory neurone in the reticular formation (co-ordinates, A.P. − 13, lateral 2·6, depth 4·45 mm), lower trace whole phrenic nerve discharge. Middle trace carotid sinus balloon pressure, which was raised at the middle of the second inspiratory burst. *t* = 500 msec.

(e.g. Fig. 7 A) was depressed for periods of 20–30 msec by a single electrical shock to sinus and IX (Fig. 7 B to D). A second type of unit had a discharge that was not so clearly locked to the expiratory phase but reached a peak frequency just prior to the beginning of inspiration and then declined as inspiration progressed. Cells of this type were excited by stimulation of the nerves as shown for sinus, IX, aortic, in Fig. 8. So far as we could tell by extracellular recording of spontaneous activity, some expiratory units
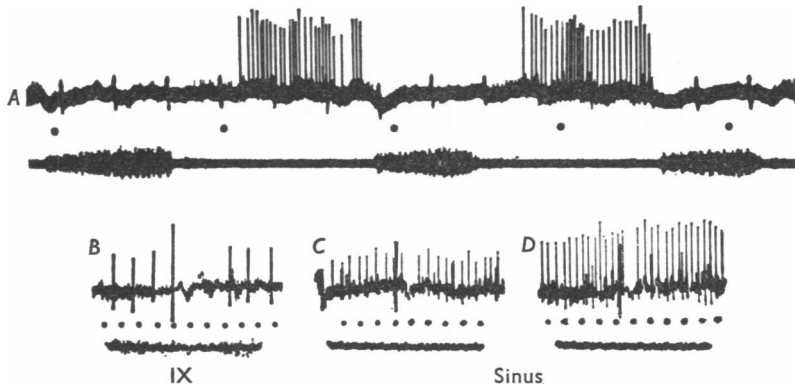

Fig. 7. Cat, Dial-urethane, artificial ventilation. In each set of records the upper trace is a micro-electrode recording of an expiratory neurone (co-ordinates, A.P. − 14, lateral 3·4, depth 1·5 mm), the lower trace is the whole phrenic nerve discharge. A, spontaneous discharge; B, with IX stimulation; C, D, with sinus nerve stimulation. $t = 1$ sec in A, 10 msec in B, 20 msec in C, D.
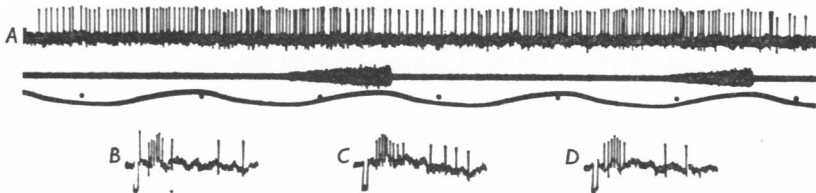

Fig. 8. Cat, decerebrate, artificial ventilation. A, upper trace is micro-electrode record from neurone in brain stem (co-ordinates, A.P. − 8, lateral 2·5, depth 6·7 mm), middle trace is whole phrenic nerve discharge, lower trace indicates phase of the artificial ventilation, expiration upwards; B, sinus nerve stimulation; C, glossopharyngeal nerve stimulation; D, aortic nerve stimulation. Time scale in A is 1 sec. Calibration signal in B to D is 2 msec and 200 μV.

were unaffected by electrical stimulation of these nerves, and none was affected by a change in sinus pressure.

Changes in membrane potential of phrenic motoneurones

Intracellular recordings were made from fifty-seven phrenic motoneurones. They were located chiefly in the third and fourth cervical segments and were

identified by antidromic invasion on stimulation of the phrenic nerve. When phrenic motoneurones were penetrated with the micro-electrode, the central phrenic depolarizing potential (CPDP) was usually recorded. When this potential reached a sufficient level the cell discharged (Fig. 9*A*). At other times the CPDP was not of sufficient magnitude to evoke action potentials (Fig. 9*B*). Hyper-ventilation was associated with an increase in the number of moto-neurones which did not discharge action potentials and sometimes was associated with complete suppression of the CPDP.

The effects of nerve stimulation. A single electrical stimulus to IX, sinus, SLN or aortic nerves evoked a hyperpolarizing potential in phrenic moto-neurones during, but only rarely in the intervals between, the variations
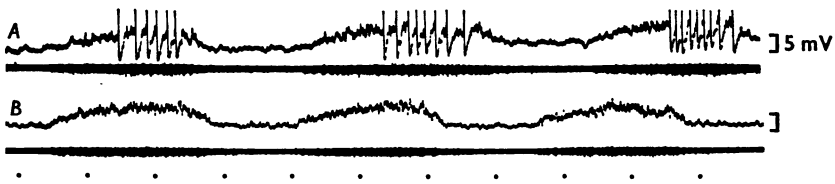

Fig. 9. Cat, Dial-urethane, artificial ventilation. The upper trace in A and B is an intracellular micro-electrode recording from a phrenic motoneurone showing the central phrenic depolarizing potential at two different times about 5 min apart. The lower trace is the whole contralateral phrenic nerve activity. Mean membrane potential 68 mV. Voltage calibration applies to upper trace and is 5 mV. $t = 500$ msec.

in CPDP. Evidence for depolarization as well as hyperpolarization of phrenic motoneurones was obtained in some experiments. Some of the results from one experiment are illustrated in Fig. 10. Stimulation of IX and sinus at low voltage (Fig. 10*A, H*), evoked a hyperpolarizing potential associated with depression of the discharge recorded from the contralateral phrenic nerve. These effects on phrenic nerve discharge had the same latency and duration as described above, i.e. 5–10 msec and 20–30 msec respectively. The hyperpolarization had a similar latency, a time to peak of 10 msec and it decayed over a further 20 msec. The amplitude of the hyperpolarizing potential was 5 mV. When the stimulus intensity was increased in this experiment instead of hyperpolarization a depolarization was evoked. This depolarization had a latency of 15–18 msec, and reached a peak after 30 msec. It was followed by a slower hyperpolarization, some 40 msec after the stimulus artifact which reached a peak 20 msec later and declined over a further 30–40 msec; appropriate changes occurred in contralateral phrenic nerve activity in phase with the changes in membrane potential (Fig. 10*B, C, I, J*). When the stimuli were applied between phrenic bursts the lower voltage had no effect (Fig. 10*D, K*), whereas the higher voltage

evoked only a depolarization in phase with a burst of phrenic activity (Fig. 10E to G and L to N). This depolarization had a latency of 10–15 msec, a time to peak of 20–30 msec and decayed over a further
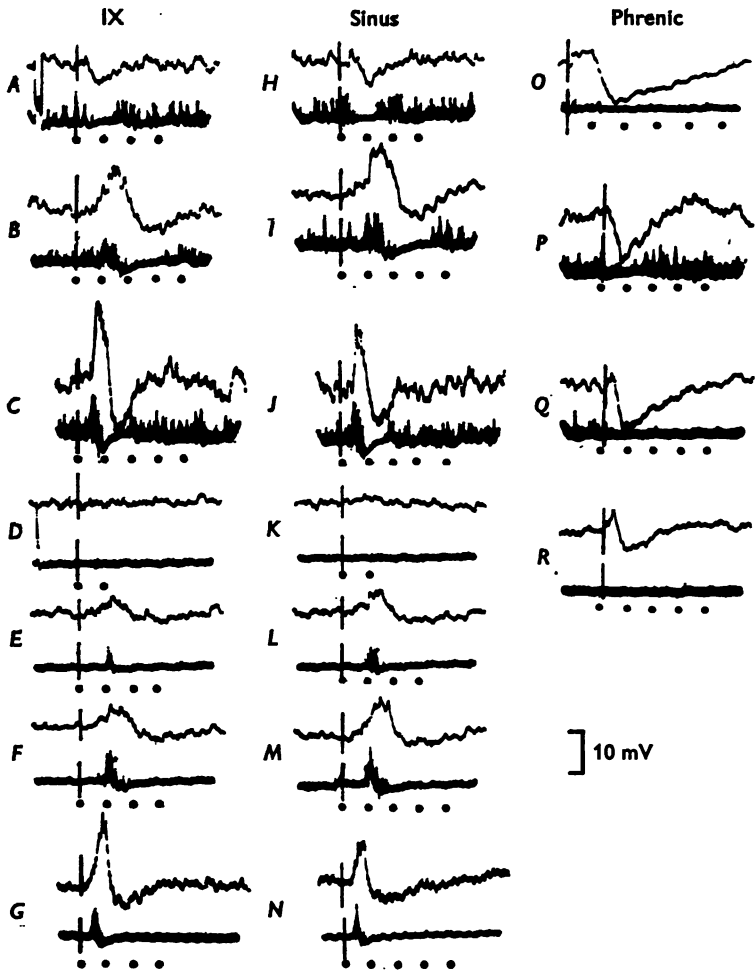

Fig. 10. Cat, Dial-urethane, artificial ventilation. In each record the upper trace is from an intracellular micro-electrode recording from a phrenic motoneurone. The lower trace is the whole contralateral phrenic nerve activity. A to G, glossopharyngeal stimulation; H to N, sinus nerve stimulation; O to R, phrenic nerve stimulation, dorsal roots intact. A to C, H to J and P, stimulation during inspiratory burst. A, H, at low intensity; B, C, I, J, at high intensity; D to G, K to N, O, R, stimulation between inspiratory bursts. D, K, at low intensity; E to G, L to N at high intensity. Q at the end of an inspiratory burst. Voltage calibration applies to upper trace and is 10 mV. Time scales are 10 msec for O, 40 msec for C, G, J, N, and 20 msec for all others.

20–30 msec; its amplitude varied between 5 and 15 mV. The depolarization is again followed by a slower hyperpolarization, (F, G, M, N), having a similar time course to that seen in inspiration. On some occasions the depolarization evoked by stimulation of the nerves was sufficient to cause the cell to discharge.

Stimulation of the ipsilateral phrenic nerve also evoked a hyperpolarization in three cells when the dorsal roots were intact. Gill & Kuno, (1963b) recorded hyperpolarization only when stimulating the contralateral phrenic nerve. The most pronounced changes were seen in the experiment illustrated in Fig. 10 and are shown between inspiratory bursts (O, R), immediately at the end of a burst (Q), and during a burst (P). In the latter case the contralateral phrenic discharge was also depressed. This hyperpolarization evoked by stimulation of the ipsilateral phrenic nerve was larger than that from the IX and sinus, amplitude 10 mV, and in Fig. 10 has a typical latency of 8 msec, a time to peak of 14 msec and a decay over 40–50 msec.

Gill & Kuno (1963b) suggested that periodic phrenic activity is controlled only by synaptic activation during the inspiratory phase. This conclusion was based on the absence of synaptic noise during the expiratory phase though their figures are not entirely convincing on this point. Subsequently Sears (1964) found that intercostal motoneurones are probably hyperpolarized between the phrenic bursts. If, in fact, phrenic motoneurones are actively hyperpolarized in the intervals between the CPDP, then it is possible that stimulation of sinus and IX would not cause further hyperpolarization at this time (Fig. 10D, K), since the membrane potential would already have moved towards the equilibrium potential for the inhibitory transmitter. Alternatively, since the cells are partially depolarized during the CPDP, the apparent hyperpolarization which we have recorded may be nothing more than a depression of CPDP and not an active hyperpolarizing process at the phrenic motoneurone membrane (see above). This point was investigated in two ways.

First, the cats were hyperventilated so that the CPDP was suppressed; electrical stimulation of the nerves at this time sometimes evoked a hyperpolarization; this is shown in Fig. 11A to E for sinus and IX. Secondly, DC currents or pulses were passed through the electrode so as to change the membrane potential; the effect on the evoked hyperpolarization was observed. The constantly changing membrane potential due to the CPDP makes this type of experiment difficult in phrenic motoneurones since the base line is very unstable; much more so than in motoneurones at other sites. Thus, many cells prove to be unsuitable and seventeen cells were tested. The average membrane resistance for six cells calculated from the current pulse–voltage deflexion was 3·15 MΩ with

a range of 2·0–6·0 MΩ. (Gill & Kuno (1963a) found an average for four cells of 3·3 MΩ.) The hyperpolarization evoked by each of the nerves was neither reversed by hyperpolarizing currents nor increased in size when the cell was depolarized. Pulses of 5–20 msec duration and 2–10 μA amplitude were used and care was taken to ensure that a plateau of current and voltage was obtained. The recorded voltage changes did not show either a
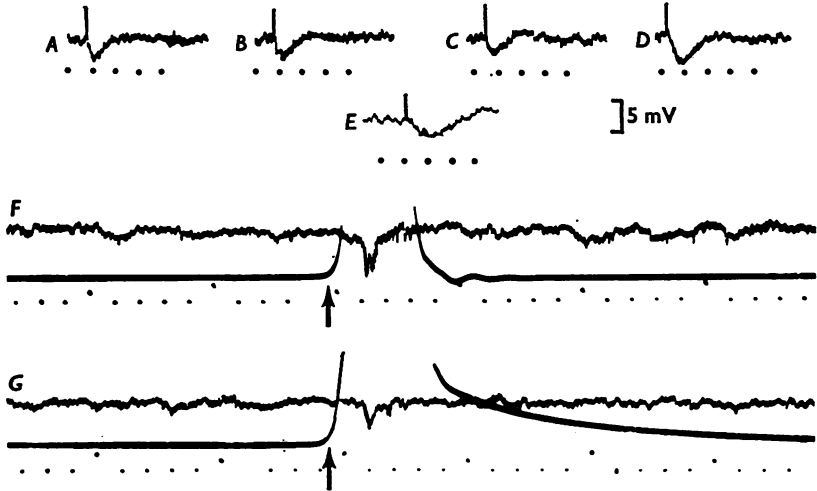

Fig. 11. Cat, Dial-urethane, artificial ventilation. Intracellular micro-electrode recording from phrenic motoneurone. Hyperpolarization evoked by sinus nerve stimulation in A, B and E, by glossopharyngeal stimulation in C, D and by raising the pressure in the carotid sinus, F, G. The lower trace in F, G, is the carotid sinus pressure, the rise commencing at the arrow. The animal was hyperventilated to eliminate the central phrenic depolarizing potentials. Time scale is 100 msec except in E where it is 10 msec.

decrease or an increase in amplitude during the period of hyperpolarization evoked by nerve stimulation; some examples of this are shown in Fig. 12. Results with the IX nerve are shown in ABC where the cell was discharging rhythmically. In A the frequency is highest and the hyperpolarization and inhibition may be seen. The hyperpolarizing pulses (5 msec duration 1 nA, recorded voltage deflexion 3 mV) are difficult to discern on the reproduced record but are clearer in B where the driven frequency of discharge of the cell is lower. In C, between the CPDPs there is no hyperpolarization. Some of the responses of another cell to SLN stimulation are illustrated in D to I. The inhibition of spontaneous discharge of the cell is clear in D. Depolarizing pulses (5 msec duration, 2 nA, recorded voltage deflexion 6·5 mV) in E are during the CPDP and in F in the interval between CPDPs. Hyperpolarizing pulses were delivered in

G to I and the result is the same as for IX nerve in A to C, namely no consistent change in pulse amplitude. Records from a third cell in J to L show in J the hyperpolarization evoked by SLN and in K, at a lower gain, superimposed 6 nA pulses, 10 msec duration, recorded voltage change = 14·5 mV. In L 1 nA pulses were delivered during IX stimulation with a
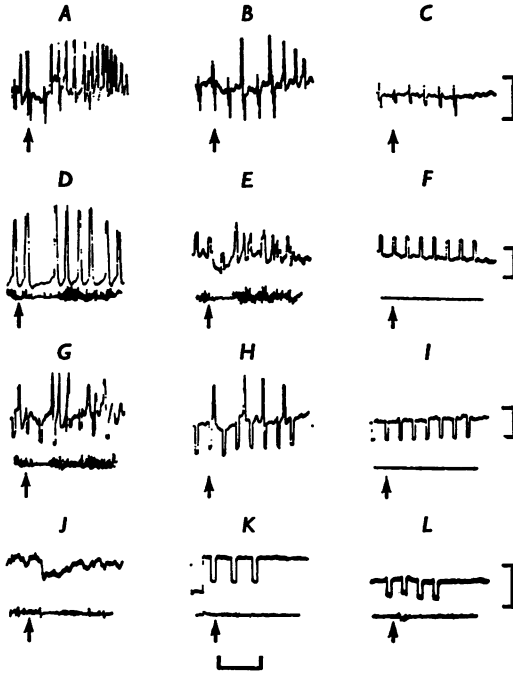

Fig. 12. The measurement of membrane conductance in three phrenic moto-neurones. Upper trace is of membrane potential, lower trace where present is contralateral phrenic discharge. Arrows mark the stimulus artifact. Current pulses were passed through the electrode and the evoked potential across the membrane recorded. In *ABCL* IX nerve and in D to K SLN was stimulated. Membrane potential in A to C 50 mV, D to I 55 mV, J to L 70 mV. Voltage calibrations to the right refer to each row and are 10 mV for all records except K and L, where the calibration to the right of L is 24 mV for K and 8 mV for L. Time scale 80 msec except in D = 40 msec. Calibration pulse at beginning of K = 20 mV and 20 msec. For full description see text.

recorded voltage change of 2·8 mV. Although with some of the cells there were occasional records where the pulses were diminished during the hyperpolarization, there was no consistent evidence of a change in any experiment. It is concluded that there is no appreciable increase in membrane conductance during the hyperpolarization and that changes in

membrane potential seen are not due to direct synaptic action. The evoked hyperpolarization from these cranial nerve afferents is then, rather, a disfacilitation (Llinas, 1964).

The effect of raising the carotid sinus pressure. In all tests cells were examined in hyperventilated cats where the CPDP was suppressed. Effects of increasing the pressure in the carotid sinus were studied on a large number of cells, but this manoeuvre commonly introduced sufficient movement to disturb the recording conditions. Therefore, results were
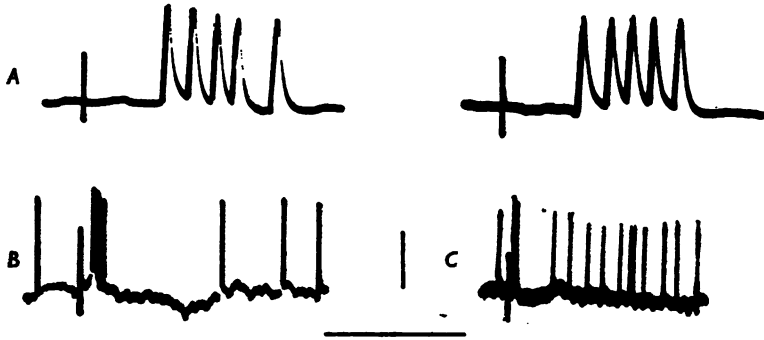

Fig. 13. Cat, Dial-urethane, artificial ventilation. Extracellular recording of a dorsal horn cell evoked by stimulation of the ipsilateral phrenic nerve. Time scale 10 msec in *A*, 100 msec in *B*, 250 msec in *C*. Voltage calibration = 5 mV.

obtained on only four cells which were not lost when the pressure was raised. The response was always a hyperpolarization lasting up to 100 msec. We did not see movement of the potential in the direction of depolarization. The best example of this response is shown in Fig. 11, *F* and *G*, where the effects of two pressure pulses are shown. This test was repeated ten times in this cell with the same results.

Spinal interneurone

Spinal interneurones excited by phrenic nerve stimulation were found in the ipsilateral dorsal horn when the dorsal roots were intact. The interneurones usually discharged with a high frequency burst in response to a single maximal shock and, if firing spontaneously, were depressed following this burst (Fig. 13). No interneurones were excited or depressed by stimulation of sinus, IX and SLN.

DISCUSSION

Gill & Kuno (1963b) showed that electrical stimulation of the reticular formation evoked excitatory and inhibitory post-synaptic potentials (EPSP, IPSP) in phrenic motoneurones, depending upon the position of the stimulating electrode. We have shown that some afferent nerves whose terminations are in the brain stem (Humphrey, 1966, 1967; Miura & Reis, 1968; Crill & Reis, 1968; Porter, 1963; Anderson & Berry, 1956; Rudomin, 1967; Biscoe & Sampson, 1970a, b), can also evoke depolarizing and hyperpolarizing potentials in phrenic motoneurones.

The nature of the early hyperpolarization has been the problem of prime interest to us. The results suggest that this potential change is not due to a direct inhibitory synaptic action on the phrenic motoneurone, since we have failed to show either that the membrane conductance changes or that the hyperpolarization reverses when the cell membrane potential is raised. In addition the hyperpolarization is unusual during the intervals between the CPDPs. At such a time the motoneurones may not be already hyperpolarized according to Gill & Kuno (1963b) (though see also Sears, 1964 and above). Thus the hyperpolarization evoked by cranial nerve afferents would appear to be subserved by a different mechanism to that operating for the hyperpolarization identified as an inhibitory post-synaptic potential by Gill & Kuno (1963b). The inhibition of phrenic motoneurones reported here is akin to the hyperpolarizing potentials investigated by Llinas (1964) in lumbar extensor motoneurones and shown by him to arise by cerebellar suppression of a reticular excitatory background activity. A possible role for the cerebellum in the inhibition described here has not yet been investigated, though Decima & Euler (1969) have shown that cerebellar stimulation may depress phrenic activity.

The means by which the suppression of phrenic motoneuronal activity comes about is complex; there is concurrent depression of so called inspiratory neurones in the brain stem. The latency of this inhibitory effect is difficult to determine when recordings are made extracellularly from cells which have a relatively slow discharge rate. It is therefore not possible to say that the time course of inhibition of brain stem neurones is appropriate to explain the inhibition of phrenic neurones by a mechanism acting through the CPDP. The CPDP would presumably be diminished if the 'inspiratory' neurones were depressed, assuming always that 'inspiratory' neurones generate it.

Since some 'expiratory' interneurones were excited and others depressed by peripheral nerve stimulation, it is difficult at present to elucidate their role in phrenic inhibition, though this finding may suggest a classification of expiratory interneurones on a functional basis rather

than the more usual anatomical one. There are also many interneurones in the nucleus reticularis parvocellularis and in the nucleus of the tractus solitarius which are evoked by stimulation of the nerves studied here (Humphrey, 1966, 1967; Sampson & Biscoe, 1968; Biscoe & Sampson, 1970*a, b*; Porter, 1963; Anderson & Berry, 1956) and whose activity is not obviously locked to respiration. In addition, some of these neurones are activated by a rise in carotid sinus pressure (Humphrey, 1966, 1967; Biscoe & Sampson, 1970*b*) whilst expiratory neurones do not seem to be so affected. Since we have shown that a rise in sinus pressure will depress phrenic activity and indeed may evoke hyperpolarization in the phrenic motoneurones, it is possible that some of the apparently non-rhythmically discharging cells in the lateral reticular formation may be implicated as interneurones in the inhibitory pathway.

The position of the descending tracts concerned with these reflexes is not clear but according to the work of Biscoe & Sampson (1970*a*) possible sites would be the medial reticulospinal tract and the dorso-lateral reticular formation.

The excitation of phrenic motoneurones, as indicated by evoked EPSPs (Fig. 10) is rather difficult to explain since the brain stem mechanisms are ill understood, but the pathway studied by Gill & Kuno (1963*b*) may be involved.

The modalities concerned in the production of the effects described have not been fully investigated but it would seem reasonable that those conveyed by IX and SLN would be concerned with touch (Sampson & Eyzaguirre, 1963). The results obtained with the study of the sinus nerve suggest that the inhibition may be a baroreceptor phenomenon and excitation may be related to chemoreceptor afferents. The same may also apply to the aortic nerve.

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