J. Physiol. (1986), **380**, *pp.* 349–371
With 10 *text-figures*
Printed in Great Britain

RESPONSES OF PHRENIC MOTONEURONES OF THE CAT TO STIMULATION OF MEDULLARY RAPHE NUCLEI

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(*Received 30 September 1985*)

SUMMARY

1. Responses of phrenic motoneurones to stimulation of the three medullary raphe nuclei (raphe magnus (r. magnus), raphe obscurus (r. obscurus) and raphe pallidus (r. pallidus)) were recorded in anaesthetized and decerebrated cats.

2. Stimulation of r. magnus or r. obscurus depressed phrenic motoneurones. Stimulation at 100 Hz reduced action potential frequency within each inspiratory burst, without appreciable changes in inspiratory duration, or number of inspiratory bursts per unit time. The depression was proportional to the stimulus intensity (40–160 μA) and frequency (12–100 Hz) and lasted throughout the period of stimulation.

3. Intracellular recording revealed concomitant depression of central respiratory drive potentials (c.r.d.p.s) and increased membrane input resistance during r. obscurus or r. magnus stimulation. In motoneurones which discharged action potentials during expiratory as well as inspiratory phases following intracellular chloride injection, stimulation of r. magnus or r. obscurus depressed cell firing during both phases. Both c.r.d.p.s and reversed inhibitory post-synaptic potentials (i.p.s.p.s) were depressed. These findings indicate that the depression is not related to post-synaptic inhibition of phrenic motoneurones.

4. Stimulation (100 Hz) of r. pallidus produced discharges of action potentials in phrenic motoneurones. Stimulation lengthened the duration of each inspiratory discharge in proportion to stimulus intensity. Continuous firing occurred throughout the period of stimulation with maximal intensities. Intracellular recordings revealed sustained depolarization and reduction in membrane input resistance during the discharge.

5. Responses were recorded extracellularly from medullary inspiratory neurones of the dorsal respiratory group (d.r.g.) and ventral respiratory group (v.r.g.) and from vagal axons which fired in phase with phrenic nerve activity. Responses to raphe stimulation were similar to those recorded from phrenic motoneurones. Evidence is presented that the responses are not related to stimulation of decussating bulbo-spinal axons from d.r.g. or v.r.g. neurones. It is suggested that medullary respiratory neurones receive inhibitory and excitatory synaptic inputs from medullary raphe neurones.

6. Hypercapnia (5 % CO_2 in O_2) or hypoxia (15 % O_2 in N_2) reduced markedly the inhibition produced during stimulation of r. obscurus or r. magnus, and restored

expiratory-linked silent periods during stimulation of r. pallidus. Activation of Hering–Breuer or baroreceptor reflexes did not alter responses to r. pallidus stimulation. Results are attributed to differences in the relative efficacies of synaptic inputs to phrenic motoneurones.

INTRODUCTION

Many regions of the medulla and pons have been investigated extensively for their potential involvement in the control of respiration. Within certain regions, for example in the dorsal (nucleus of the solitary tract; n.t.s.) and ventral (nucleus retroambigualis, n.r.a.; nucleus ambiguous, n.a.) respiratory groups, neurones exhibit patterns of excitation and inhibition which suggest that they are part of the neural network involved in breath-to-breath control of respiration (Merrill, 1974; Cohen, 1979; Richter, 1982; Ballantyne & Richter, 1984; Bianchi, 1985). However, additional important contributions to respiratory control may be made by cortical, hypothalamic and brain-stem reticular neurones, which influence patterns of respiration to adjust for altered state of consciousness, changes in ambient temperature, increased O_2 demand, and for reflex acts associated with speech, swallowing, etc. (Pitts, Magoun & Ranson, 1939; Redgate & Gellhorn, 1958; Sumi, 1964; Wang & Ngai, 1973; Harper, Frysinger, Marks, Zhang & Frostig, 1985). Neurones in the latter category generally do not exhibit respiratory-related discharge patterns, however their ability to alter respiration can be demonstrated. During electrical stimulation of relatively circumscribed regions of the medullary reticular formation, powerful and sustained changes in respiration are produced (Pitts *et al.* 1939; Anderson & Sears, 1970). Pitts and co-workers (Pitts *et al.* 1939) demonstrated that stimulation of the mid line reticular formation of the medulla rostral to the obex, in the regions now referred to as raphe magnus and raphe obscurus (Taber, Brodal & Walberg, 1961), produced cessation of breathing, whereas stimulation of raphe pallidus increased ventilatory effort markedly. Caudal to the obex, sites of excitation and inhibition were found in close association within raphe obscurus. In a later investigation of raphe neurones, Sessle, Ball & Lucier (1981) showed that certain respiratory reflexes were abolished during stimulation of raphe magnus, while respiration-related discharges of neurones in the nucleus of the solitary tract were weakly depressed. These studies, which indicate that the three components of the caudal raphe complex have powerful, differential effects on respiration, prompted the investigation reported here.

In the present investigation, responses of respiratory neurones, particularly phrenic motoneurones, to stimulation of raphe magnus (r. magnus), raphe obscurus (r. obscurus) and raphe pallidus (r. pallidus) are examined. Along with the demonstration of dramatic responses evoked by stimulation, an attempt is made to determine if the primary influences occur at spinal or supraspinal sites. An additional objective was to obtain an estimate of the relative efficacy of the raphe input to phrenic motoneurones. This was done by determining whether or not the effects of raphe stimulation would be altered during activation of chemoreceptor, baroreceptor or vagal pulmonary afferent fibres. These afferents can exert considerable influence on the spontaneous discharge patterns of respiratory neurones (Cohen, 1969; Clark & Von Euler, 1972; Richter & Seller, 1975; Lipski, McAllen & Spyer, 1977; Bainton, Kirkwood & Sears, 1978; Bainton & Kirkwood, 1979). It was therefore of interest

to determine if phrenic nerve responses to raphe stimulation would also be altered. Portions of this investigation have been reported in preliminary form (Lalley, 1984).

METHODS

Anaesthesia and preparation of cats

Sixty-four cats of either sex, weighing between 2·3 and 4·8 kg, were used to obtain data for parts of this and another study (Lalley, 1986). In five experiments, cats were decerebrated by blunt transection at the midcollicular level, followed by removal of the cerebellum and all brain tissue rostral to the transection. The remaining experiments were performed on cats with intact neuroaxis, which were anaesthetized with sodium pentobarbitone (40 mg/kg I.P. initially, supplemented by 4 mg/kg I.V. every hour), or with combinations of α-chloralose and urethane (50 and 250 mg/kg I.V., respectively) or diallylbarbituric acid, monoethylurea and urethane (80, 280 and 280 mg/kg I.P., respectively).

Polyethylene catheters were inserted in the radial vein for administration of drugs, and in the femoral artery in order to record systemic arterial pressure. A glass cannula was inserted in the trachea, close to the clavicle, through a mid line ventral incision. The cervical spinal cord (C3–C7) and fifth cervical branch of the phrenic nerve were exposed through a dorsal mid line incision. The cervical vagus nerve was also exposed in some experiments. The nerve trunk was sectioned and the central end was desheathed and mounted on a bipolar silver hook electrode for recording or stimulation. The head of the cat was fixed in a steriotaxic frame. Clamps attached to the T1 and L5 or L6 superior spinous processes were used to suspend the animal in a spinal frame attached to the steriotaxic apparatus. The dorsal surface of the medulla was exposed by ventroflexing the head and removing the occipital bone and the cerebellum caudal to the cerebellar peduncles.

All exposed tissue was covered with paraffin oil, which was maintained at a temperature of 37–39 °C by heating coils. Body temperature was also maintained at 37–39 °C by external heating devices. The animals were paralysed with pancuronium bromide (200 μg/kg I.V. initially, 75 μg/kg every 30 min thereafter) and ventilated by a respiratory pump. In experiments which required intracellular recording from phrenic motoneurones, bilateral thoracotomy was performed. End-tidal CO_2 was maintained (LB-1 medical gas analyser, Beckman, U.S.A.) between 4·0 and 4·6% by adjusting the stroke volume or rate of the pump.

Recording and stimulation procedures

Methods similar to those described previously (Biscoe & Sampson, 1970; Berger, 1979; Lalley, 1983) were used to stabilize the spinal cord for intracellular recording. During recording from medullary respiratory neurones, pulsations were reduced with a pressure foot placed lightly on the surface of the medulla. Glass micropipettes (10–20 MΩ d.c. resistance) filled with 3 M-KCl or 4 M-potassium acetate were used to record from phrenic motoneurones or medullary neurones. Phrenic motoneurones were identified by the occurrence of antidromically conducted action potentials evoked by stimulating the phrenic nerve. Medullary units of the dorsal respiratory group (d.r.g.), ventral respiratory group (v.r.g.) or Botzinger complex were classified as inspiratory or expiratory neurones from the timing of action potentials in phase or out of phase, respectively, with phrenic nerve activity. Intracellular potentials were detected with a d.c. electrometer (model 8500, Dagan Corporation, U.S.A.). Action potentials recorded from the phrenic and vagus nerves were amplified by a.c. pre-amplifiers (model P511, Grass Instruments, U.S.A., 10–3000 Hz band width), displayed along with intracellular potentials on an oscilloscope (model 5100, Tektronix, U.S.A.) and photographed (C5 kymographic camera, Grass Instruments, U.S.A.): potentials were stored on magnetic tape (model B recorder, Vettor Corporation, U.S.A.) for further analysis. Action potentials were led off to window discriminator–rate-meters (constructed by the Medical Electronics Laboratory, University of Wisconsin, U.S.A.) and recorded on a polygraph (model 6, Gilson Medical Electronics, U.S.A.) as an analog voltage which varied with instantaneous discharge frequency.

Rectangular constant current pulses from a stimulator (model S-88, Grass Instruments, U.S.A.) were applied to sites in the caudal raphe complex through bipolar concentric electrodes (No. SNE-100, Rhodes Medical Instruments, U.S.A.) which had inside and outside diameters of 100 and 250 μm, respectively. The inner pole served as the cathode. Reactive sites in the caudal raphe complex, from a plane 6 mm rostral to the obex to 1 mm caudal, were sought. The electrode was

inserted along the mid line, normal to the dorsal surface of the medulla for all but the most rostral tracks, which sometimes required a forward angle of 15–25 deg. The electrode in each track was advanced vertically in 0·5 mm steps. At each depth, 100 Hz, 0·05 ms constant current pulses were applied for 20–30 s at intensities of 10 to 400 μA. In this manner, sites which produced maximal inhibitory or excitatory effects on phrenic or vagus nerve activity were detected. The most sensitive sites were subjected to a range of current intensities and varying stimulus parameters (single shocks, brief trains, 100 Hz stimulation for 30 s periods).

Activation of chemoreceptors, baroreceptors and Hering–Breuer reflex afferents

Chemoreceptors were stimulated by administering 15 % O_2 in N_2 or 5 % CO_2 in balanced O_2 from 5 l plastic bags which were connected via polyethylene tubing to the input port of a respiratory pump (model 702, Harvard Apparatus, U.S.A.). The Hering–Breuer inspiratory-inhibiting reflex was activated by adding a volume of air from a 30 ml syringe to the stroke volume of the pump just before the inflation phase. Baroreceptor reflexes were activated by elevating blood pressure with intravenous administration of a pressor agent (metaraminal, 20–50 μg/kg).

Histology

Identification of reactive sites in the medullary raphe complex was facilitated by deposition of iron during passage of d.c. current (0·1 mA for 30 s), and perfusion of the animal with 1 % w/v potassium ferrocyanide in formaldehyde after I.V. administration of a lethal dose of sodium pentobarbitone. The resulting Prussian Blue reaction product was located on 50 μm frozen coronal sections stained with cresyl violet. Microscopic identification of sites in the medullary raphe complex was aided by the atlas of Berman (1968) and by maps and anatomical descriptions of the raphe nuclei (Taber, 1961).

RESULTS

Response patterns recorded from phrenic nerve during raphe stimulation

Two types of altered responses in phrenic motoneurones were elicited during stimulation of the medullary raphe complex. Stimulation of r. magnus or r. obscurus reduced or abolished spontaneous respiratory discharges, whereas stimulation of r. pallidus increased firing. The responses recorded from the phrenic nerve during stimulation of r. obscurus and r. pallidus, within a track 3 mm rostral to the obex, are shown in Fig. 1.

The inhibitory response to 100 Hz stimulation of r. obscurus or r. magnus consisted of a stimulus-dependent reduction in the frequency of action potentials within each inspiratory burst. As shown in Fig. 1*A*, it was possible to abolish spontaneous discharges for the duration of stimulation with maximal stimulus intensities. At submaximal intensities of stimulation, the frequency of action potentials within each inspiratory discharge was reduced without significant change in the duration of the inspiratory discharge or the expiratory silent period. Therefore, the degree of inhibition was expressed as the percentage reduction in peak frequency of action potentials during an inspiratory discharge, for a given stimulus intensity or stimulus frequency. This criterion was used in constructing the graphs illustrated in Fig. 2.

In twelve experiments from which the data points of Fig. 2 were obtained, peak frequencies of ten consecutive inspiratory discharges were measured, before and during each stimulation, and the respective averages were used to determine the percentage inhibition produced at each intensity or frequency. Stimulus intensity is expressed in terms of multiples of threshold, the latter being defined as the lowest intensity or frequency which produced a 5 % reduction in peak action potential

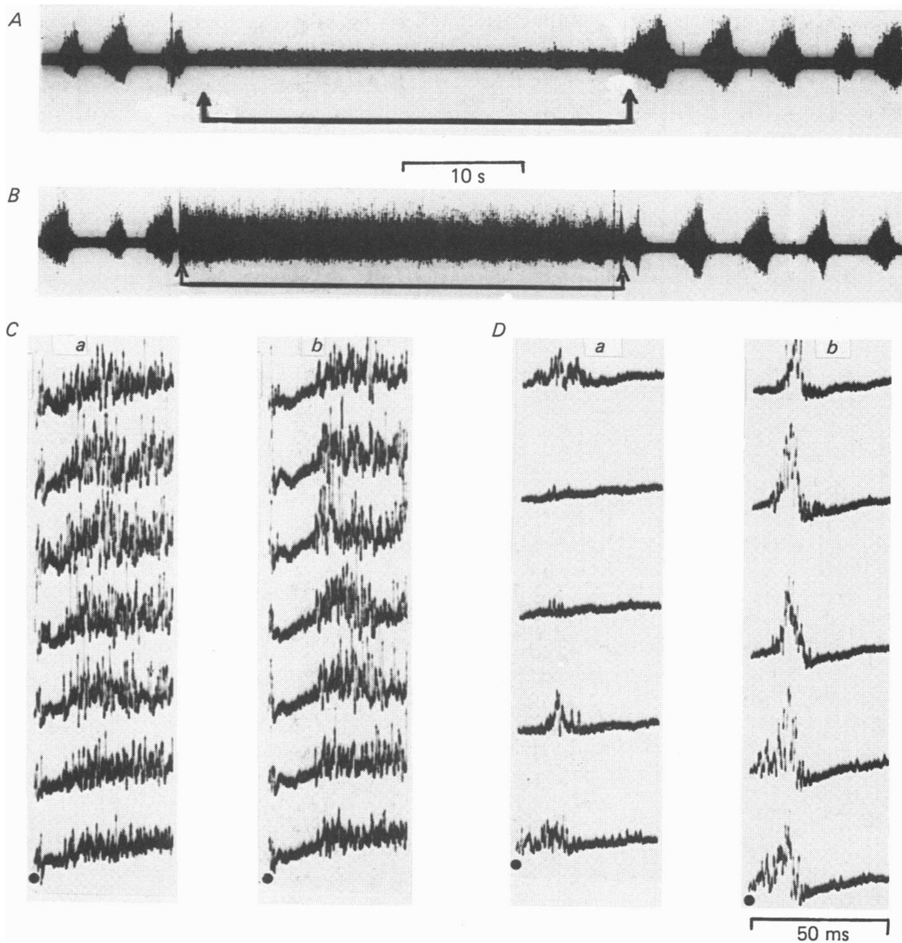

Fig. 1. Film records of responses recorded from phrenic nerve to stimulation of r. obscurus and r. magnus. *A*, horizontal film records of responses to stimulation of r. obscurus. During time denoted by bar and arrows, r. obscurus was stimulated with 100 μA, 0·05 ms pulses at 100 Hz frequency. *B*, responses evoked during stimulation of r. pallidus with same stimulus parameters. *C*, film records of responses evoked by five pulses, 1000 Hz, delivered to r. obscurus at the onset of each sweep. Oscilloscope sweeps, one per trace in each column, are consecutive and were triggered at a rate of 3 Hz by the stimulator. Stimulus intensities are 100 μA in *Ca*, 150 μA in *Cb*. *D*, responses evoked during stimulation of r. pallidus. Format similar to *C;* however, oscilloscope sweeps and stimulus trains were triggered at a rate of 2 Hz. *Da*, 100 μA intensity; *Db*, 150 μA intensity.

frequency. For 100 Hz pulses of 0·05 ms duration, threshold intensity was, on average, 40 μA. Complete inhibition was observed consistently when stimulus intensity was four times threshold (160 μA). As seen in Fig. 2*B*, threshold stimulus frequency (at 160 μA) was 12 Hz, while maximal inhibition occurred at 100 Hz.

Inhibition of spontaneous activity in phrenic motoneurones was also evident with single shocks or brief trains, either of which produced a silent period in the spontaneous inspiratory discharge, and thereby provided a measure of the latency for inhibition. In Fig. 1 (*Ca* and *Cb*) r. obscurus was stimulated with brief trains (five

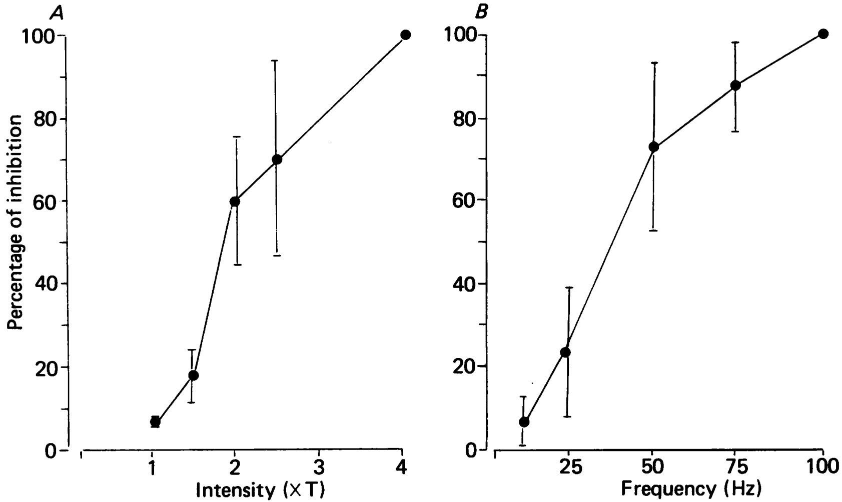

Fig. 2. Stimulus–response relationships obtained in twelve experiments during stimulation of r. obscurus. Ordinates, percentage reduction in peak firing frequency recorded from phrenic nerve. Each value was obtained by measuring peak instantaneous firing frequency before (control) and during stimulation with different stimulus parameters. Abscissa in *A*, stimulus intensity expressed as multiples of threshold (T). Raphe obscurus stimulated with pulses, 100 Hz, for 30 s during each test (T = 40·4 ± 8·8 μA at 100 Hz). Abscissa, in *B*, frequency of stimulation; stimulus intensity was four times T at 100 Hz (T = 12 Hz at this stimulus intensity). For intensity- and frequency-response relationships, threshold is defined as the intensity or frequency which produced 5% inhibition. Pulse duration in all tests was 0·05 ms. Vertical bars represent standard error.

pulses, 1000 Hz) at a rate of 3 trains/s. Traces in each column are single consecutive responses evoked during two spontaneous inspiratory discharges, one inspiratory burst in *Ca*, one burst in *Cb*. Stimuli were applied at the beginning of each oscilloscope sweep, as denoted by the filled circles at the bottom of each column. The records show inhibition of spontaneous firing which begins during, or just after, each train. In this experiment and in others, the latency for inhibition evoked by r. obscurus or r. pallidus stimulation was 4–8 ms. Increasing the stimulus intensity further lengthened the duration of inhibition (*Cb*) without altering the onset. The intensity of spontaneous firing following inhibition was equivalent to the pre-stimulus level, thus there was no evidence for a post-inhibitory rebound discharge.

Stimulus parameters which produced complete and sustained inhibition during r. obscurus stimulation also evoked modest hypotensive episodes, during which systolic blood pressure fell on the average by 15 ± 4·5 (S.E. of mean) mmHg. In three experiments, however, blood pressure was elevated by an average of 35 ± 20 mmHg. During stimulation of r. magnus, at intensities which produced complete inhibition of phrenic nerve activity, no consistent pattern of blood pressure response was evident. Pressure was elevated in some experiments, lowered in others, or responded with an initial elevation and subsequent depression. In all experiments, afferent fibres from carotid sinus and aortic arch baroreceptors were intact.

The response to stimulation of r. pallidus varied with stimulus parameters in a manner more complex than was associated with stimulation of r. obscurus or r. magnus. As illustrated in Fig. 1 *Da*, single shocks or brief trains at submaximal

intensity (60–100 μA) evoked short-latency (4–8 ms) discharges which were most intense during the early part of inspiration, and weakest, or absent, during the expiratory interval. When stimulus intensity was maximal (160–180 μA), discharges were consistently evoked, irrespective of their timing with respect to the phase of respiration, as seen in Fig. 1 *Db*. Shorter latency responses, at 3–3·5 ms latency, could sometimes be seen during the inspiratory phase, particularly with stimulus intensities greater than 180 μA. It is assumed that these responses represent activation of decussating bulbo-spinal axons which originate in the dorsal or ventral medullary inspiratory regions. During 100 Hz stimulation, increasing the intensity converted the discharge pattern from inspiratory-related bursts to sustained firing at stimulus intensities of 160–220 μA (Fig. 10 *Ba*). At lower intensities (100–160 μA) a combination of tonic and inspiratory-phased firing was observed, similar to the pattern of activity evident in the later stages of the evoked response shown in Fig. 1 *B*. The stimulus intensities necessary to evoke these responses produced changes in blood pressure in only four experiments, where increases or decreases not exceeding 20 mmHg were observed.

Responses recorded from the vagus nerve

Respiratory-linked discharges were recorded in four experiments from the central end of the cut vagus nerve. In these experiments, prominent efferent respiratory discharges in phase with phrenic nerve discharges were present. Vagal inspiratory discharges were altered by raphe stimulation in a manner identical to those of the phrenic nerve. Responses recorded concurrently from the phrenic nerve and vagus nerve are illustrated in Fig. 3. As shown by the responses to three different intensities of stimulation within r. obscurus, vagal inspiratory discharges were depressed more readily than phrenic nerve activity. At a stimulus intensity (200 μA) supramaximal for the abolition of all respiratory-related firing, elevation of blood pressure and a low-level tonic discharge of the vagus nerve are evident.

Distribution of reactive points

The medullary raphe complex was stimulated at numerous points within a region extending from 6 mm rostral to the obex to 1 mm caudal. A map of histologically identified reactive sites from seventeen experiments is illustrated in Fig. 4. At sites 5–6 mm rostral to the obex and ventral to the medial longitudinal fasciculus within the region corresponding to r. magnus, stimulation produced inhibition of firing (*A* and *B*). At more rostral and ventral points, alterations in respiratory patterns were absent. The most pronounced and consistent inhibition of spontaneous firing occurred when the electrode was 3 mm rostral to the obex (*C*). Inhibitory points were 1·5–3 mm below the dorsal surface, in an area which includes r. obscurus, and the dorsal region described by Yen & Blum (1984). Discharges of phrenic and vagal motoneurones were evoked at points 4·5–6·5 mm below the dorsal surface, as far rostral as 5 mm from the obex. This region includes r. pallidus, and possibly the most ventral part of r. obscurus. The most intense discharges were evoked 3 mm rostral to the obex (*C*). The region caudal to the obex (*F*) was stimulated in only two experiments, in which excitatory as well as inhibitory points were found in r. obscurus.

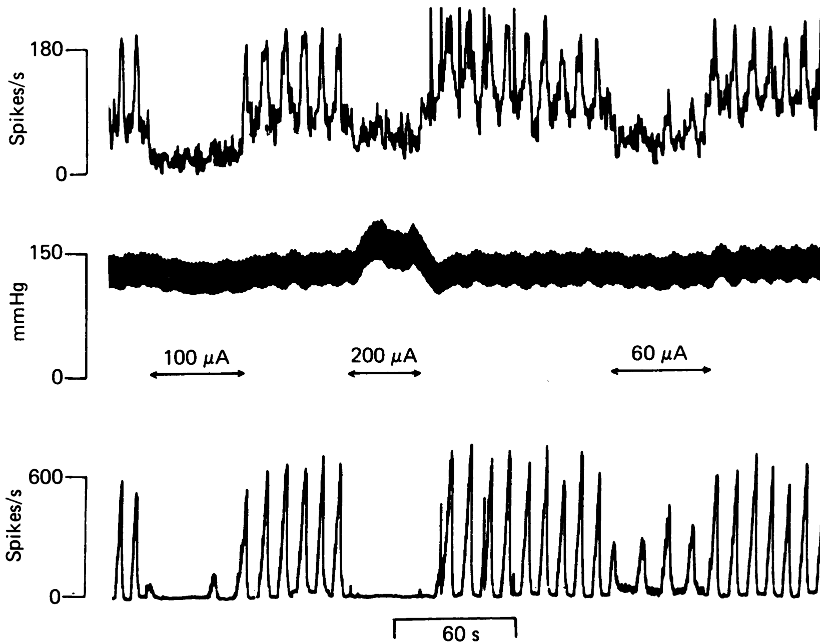

Fig. 3. Effects of stimulating r. obscurus on blood pressure and on central respiratory discharges recorded from the vagus and phrenic nerves. Top trace, rate-meter records of instantaneous firing frequency recorded from the central end of the vagus nerve. Middle trace, systemic arterial blood pressure. Lower trace, rate-meter records of phrenic nerve activity. Arrows under middle trace denote episodes of stimulation at three stimulus intensities, all at 100 Hz frequency, 0·05 ms pulse duration.

Intracellular analysis

Responses to raphe stimulation were recorded and analysed in eleven experiments, from twenty-five phrenic motoneurones which discharged action potentials with a respiratory periodicity and which had stable membrane potentials of -55 to -70 mV during expiration. Examples of responses to stimulation of r. obscurus, r. pallidus and r. magnus are seen in Fig. 5. During stimulation, of r. obscurus or r. magnus, the amplitudes of central respiratory drive potentials were reduced and the neurones stopped firing (*A*, *C*, *E* and *F*). Inhibition of firing was accompanied by an immediate increase in input resistance (*C* and *F*), which increased further with continued stimulation until a maximum level was reached after 10–12 s. The progressive increase of resistance was observed consistently during repeated tests in these experiments and in others. Augmented inspiratory discharges immediately following the cessation of stimulation are evident in *A*, *C*, *E* and *F* and were observed in recordings from other phrenic motoneurones.

Phrenic motoneurones ($n = 5$) were injected intracellularly with chloride ions in two experiments, which resulted in the reversal of expiratory inhibitory post-synaptic potentials (i.p.s.p.s). The depolarizing i.p.s.p.s were sufficient to generate action potentials, as shown in the results from one experiment depicted in Fig. 6. Control responses are shown in traces *A*–*C*. Two bursts of action potentials accompanied each

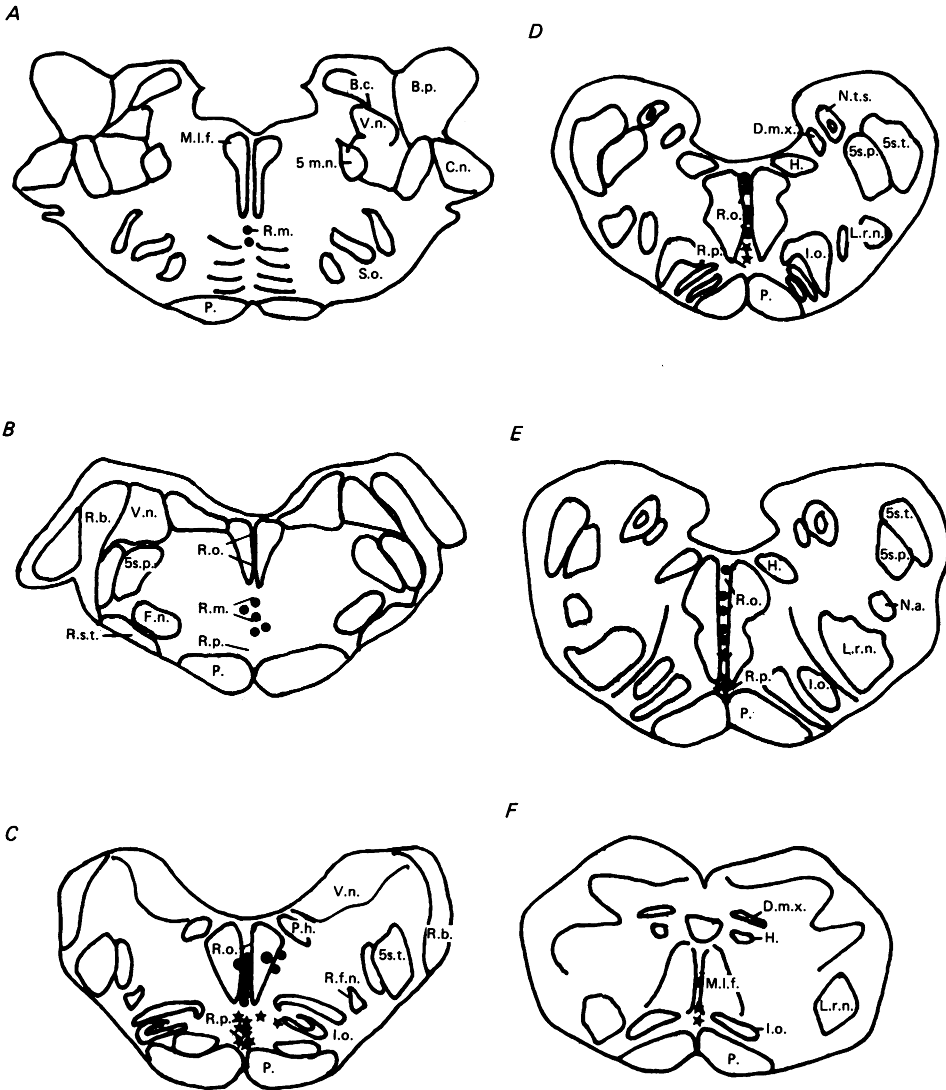

Fig. 4. Distribution of reactive sites in the raphe nuclei of the medulla. Sites at which stimulation produced depression of inspiratory discharges recorded from phrenic nerve are marked by filled circles; sites where stimulation increased discharges are denoted by stars. Drawings are of cross-sections of medulla from a level 6 mm rostral to the obex (*A*) to 1 mm caudal (*F*). Abbreviations: b.c., brachium conjunctivum; b.p., brachium pontis; c.n., cochlear nucleus; d.m.x., dorsal motor nucleus of the vagus; f.n., facial nucleus; h., hypoglossal nucleus; i.o., inferior olive; l.r.n., lateral reticular nucleus; m.l.f., medial longitudinal fasciculus; n.a., nucleus ambiguous; p., pyramidal tract; p.h., nucleus praepositus hypoglossi; r.b., restiform body; r.m., nucleus raphe magnus; r.o., nucleus raphe obscurus; r.p., nucleus raphe pallidus; r.s.t., reticulospinal tract; s.o., superior olive; v.n., vestibular nucleus; 5m.n., trigeminal motor nucleus; 5s.p., trigeminal sensory nucleus; 5s.t., trigeminal tractus spinalis.

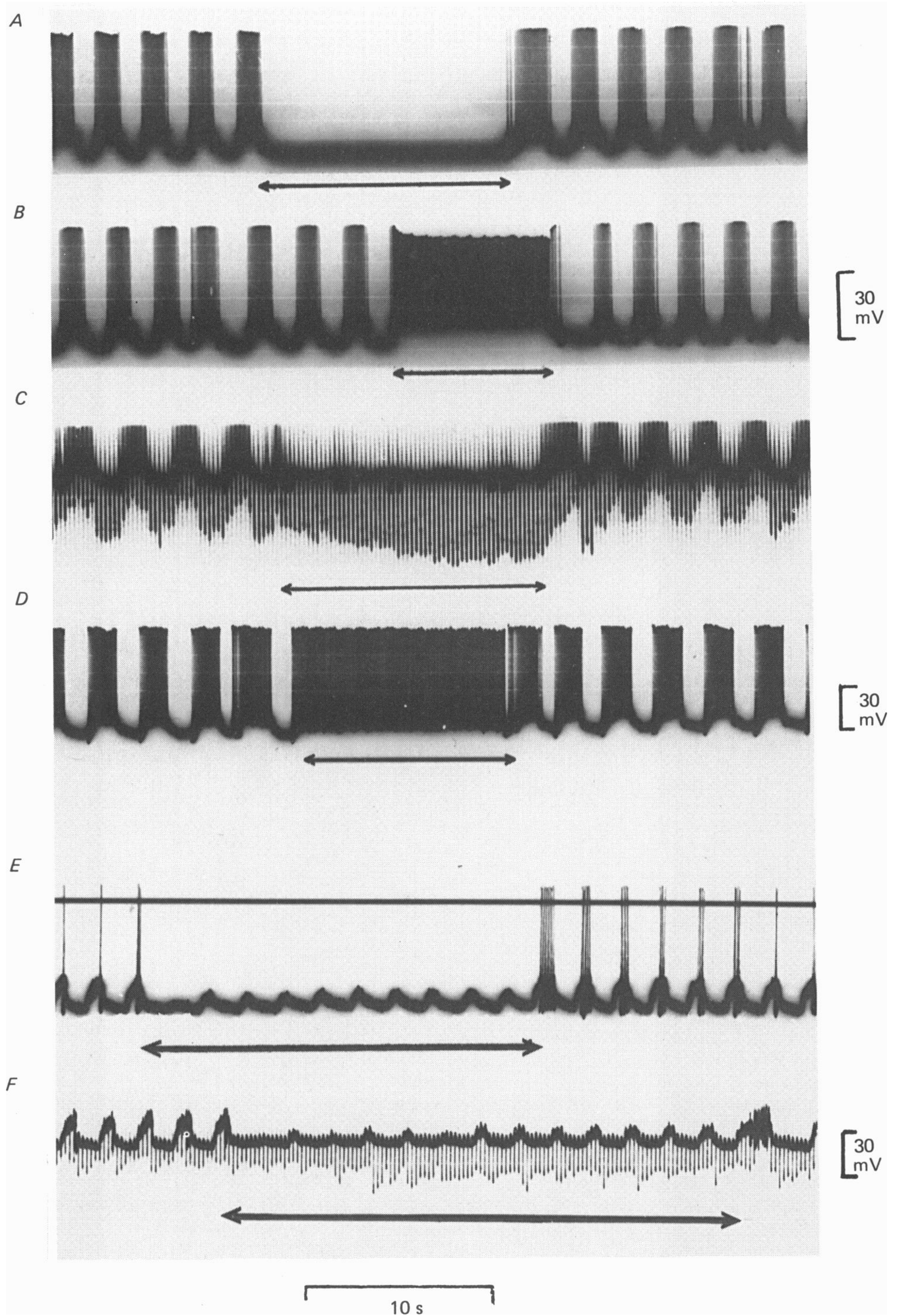

Fig. 5. For legend see opposite.

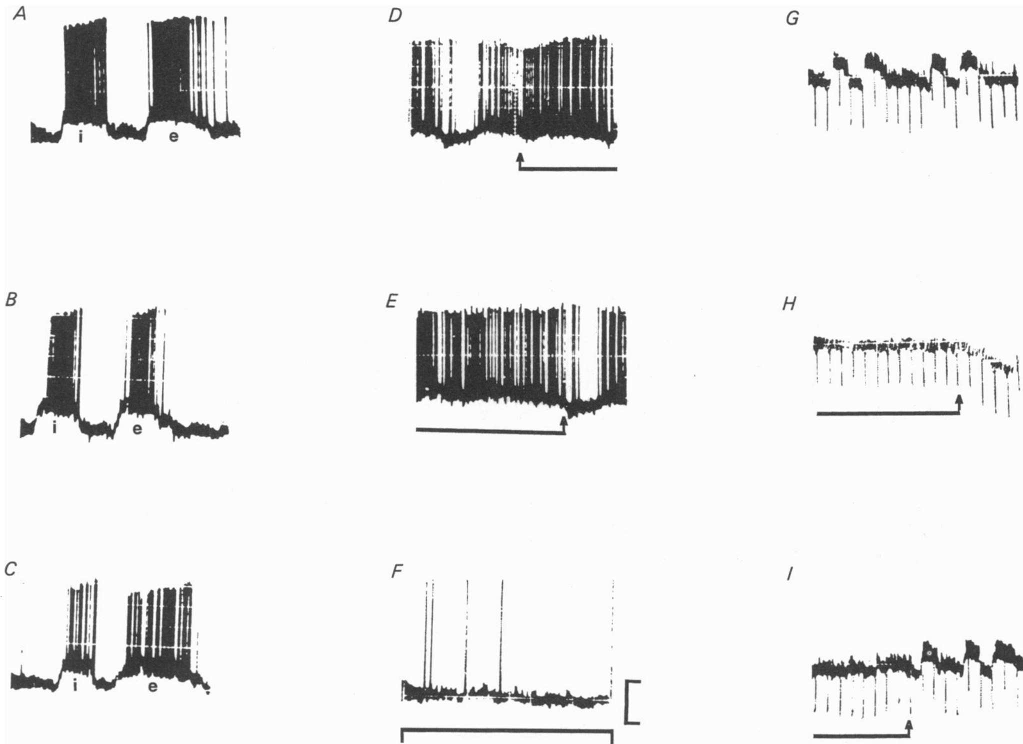

Fig. 6. Responses to raphe stimulation recorded intracellularly from a phrenic motoneurone after injection of chloride. *A–C*, control responses. First discharge (i) in each trace occurs during inspiration. Discharges labelled 'e' are due to chloride reversal of i.p.s.p.s and occur during expiration. *D* and *E*, sustained discharge evoked by r. pallidus stimulation. Stimulation begins during *D* and ends during *E*. *F*, inhibition of both inspiratory-linked and expiratory-linked discharges during r. obscurus stimulation. *G*, control response. Trace shifted upward to reveal hyperpolarizing electrotonic potentials produced by 6 nA, 20 ms current pulses. *H*, responses recorded during r. pallidus stimulation, which ends at arrow. Note depolarization and decreased input resistance during stimulation. *I*, responses to r. obscurus stimulation, which begins at arrow. Voltage calibration is 30 mV, time calibration is 200 ms for *A–F*, 500 ms for *G–F*.

Fig. 5. Film records of intracellular responses of a phrenic motoneurone to stimulation of r. obscurus, r. pallidus and r. magnus. Responses are from three motoneurones: *A* and *B*; *C* and *D*; *E* and *F*. *A*, response to 100 Hz stimulation of r. obscurus. *B*, response of same neurone to r. pallidus stimulation, at a point 5 mm ventral to the dorsal surface of the medulla. *C*, response of second neurone to r. obscurus stimulation. Trace shifted upward to reveal hyperpolarizing electrotonic potentials produced by intracellular stimulation with 6 nA, 20 ms current pulses. *D*, response of second neurone to stimulation of r. pallidus at a point 4 mm ventral to dorsal surface of medulla, in same electrode track as in *A–C*. *E*, 100 Hz stimulation of r. magnus. *F*, activity recorded during passage of 6 nA, 20 ms intracellular hyperpolarizing pulses. Action potentials not visible because of shift in base line on oscilloscope. Duration of raphe stimulation denoted under each trace by lines and arrows.

respiratory cycle after intracellular chloride ion injection. Each inspiratory-linked discharge was followed by a silent period with increased input resistance, then by a burst of action potentials arising from reversed i.p.s.p.s. During stimulation of r. obscurus, (*F* and *I*), action potentials which occurred during expiration as well as those during inspiration were depressed and, throughout the period of stimulation, input resistance was increased (*I*). Therefore it can be assumed that the inhibition produced by r. obscurus or r. magnus stimulation is not impressed directly on the soma membrane of the phrenic motoneurone. It is also evident that the inhibition influences expiratory as well as inspiratory neurones which control the spontaneous discharge patterns of phrenic motoneurones.

Stimulation of r. pallidus resulted in depolarization and generation of action potentials. The magnitude of the response depended both on the stimulus parameters and the location of the stimulating electrode. In Fig. 5*B* and *D*, responses of two motoneurones from the same experiment to r. pallidus stimulation are shown. The response illustrated in *B* was obtained during stimulation in the same track, but with the electrode 1 mm more ventral in r. pallidus than the site which produced the response in *D*. It can be seen that the level of depolarization and frequency of discharge are greater during stimulation at the more ventral site. The responses of another phrenic motoneurone to r. pallidus stimulation are illustrated in Fig. 6*D*, *E* and *H*. During the sustained depolarization evoked by 100 Hz stimulation, irrespective of depth within r. pallidus, input resistance decreased relative to the resistance associated with the expiratory phase of spontaneous firing (*H*). Stimulation of r. pallidus with brief trains or single shocks less than 200 μA evoked polysynaptic excitatory post-synaptic potentials (e.p.s.p.s) and action potentials. Earliest e.p.s.p.s appeared 4–5 ms after stimulation. Greater intensities of stimulation (200–450 μA) produced e.p.s.p.s and spikes with latencies as short as 3 ms. Responses characteristic of r. pallidus stimulation are shown in Fig. 7*C*. Although the electrode tip in this instance was located at the junction of r. pallidus and r. obscurus, the excitatory responses in *C* are identical to those which were evoked by stimulation when the electrode tip was solely within r. pallidus. Single shocks are seen to generate e.p.s.p.s which begin after 4 ms. During the inspiratory phase, the e.p.s.p.s are seen to give rise to two to three action potentials. In this experiment and others, composite responses were evoked during 100 Hz stimulation when the electrode tip was situated at the border between r. obscurus and r. pallidus, or between r. magnus and r. pallidus. During stimulation in Fig. 7*B*, spontaneous firing was initially depressed, presumably by r. obscurus stimulation, despite a sustained depolarization which arises from stimulation of r. pallidus. The resumption of firing seen after 11 s of stimulation may have been the result of the concurrent increase of about 50% in membrane input resistance, which may have reduced shunting of the depolarizing synaptic currents generated by r. pallidus stimulation.

Responses of d.r.g. and v.r.g. neurones

Extracellular recordings were obtained from neurones which exhibited respiratory discharge patterns in three regions of the medulla: d.r.g. neurones in the ventrolateral nucleus of the solitary tract, v.r.g. neurones located in nucleus retroambigualis, and v.r.g. neurones near the retrofacial nucleus (r.f.n.). Responses of these neurones to

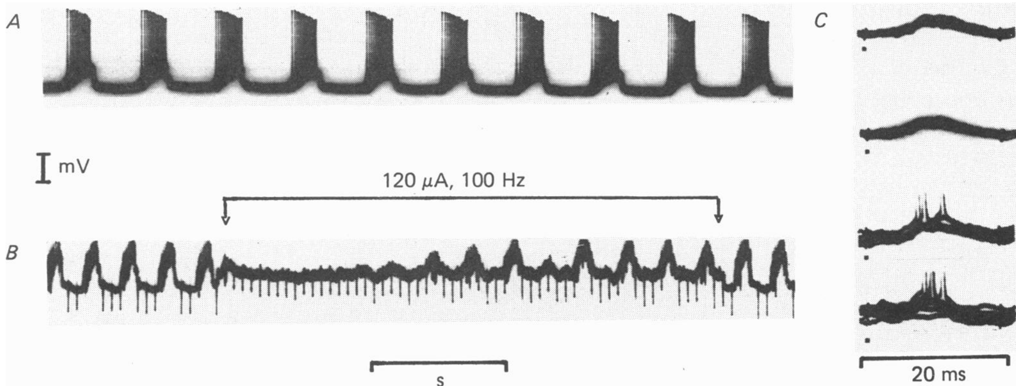

Fig. 7. Response of phrenic motoneurone to 100 Hz stimulation in medulla near the junction of r. obscurus and r. pallidus, just in front of obex. *A*, spontaneous firing. *B*, during stimulation. Base line shifted upward to show hyperpolarizing potentials produced by 6 nA, 15 ms intracellular current pulses. *C*, e.p.s.p.s evoked in the same motoneurone by single shocks, 2 Hz. Several oscilloscope sweeps superimposed in each of the four records. Stimulus artifacts denoted by filled squares under each record. Two upper records recorded during expiratory period. Time calibration is 10 s for *A*, 20 s for *B*. Voltage calibration is 30 mV for *A*, 15 mV for *B*.

raphe stimulation (40–160 μA) were recorded simultaneously with phrenic nerve activity. Since responses to raphe stimulation were produced in vagal respiratory motoneurones (Fig. 3), these experiments were performed in order to uncover evidence for an influence of the raphe complex on other medullary respiratory neurones, and to determine if responses to raphe stimulation were synaptic or antidromic. In the latter regard, antidromic responses of d.r.g. or v.r.g. neurones would indicate that effects produced on phrenic motoneurones were due, at least in part, to stimulation of d.r.g. or v.r.g. axons which pass through the raphe nuclei. From other studies it is known that inspiratory bulbo-spinal axons from d.r.g. and v.r.g. neurones cross the mid line rostral to the obex and activate phrenic and inspiratory intercostal motoneurones monosynaptically (Fedorko, Merrill & Lipski, 1983). In addition, d.r.g. neurones and phrenic motoneurones receive inhibitory monosynaptic inputs from expiratory neurones of the r.f.n.; more specifically, from those r.f.n. expiratory neurones which comprise the Botzinger complex (Merrill, Lipski, Kubin & Fedorko, 1983; Fedorko & Merrill, 1984; Merrill & Fedorko, 1984).

Medullary respiratory neurones in the present study responded synaptically, but not antidromically, to raphe stimulation. Inspiratory neurones in n.t.s. ($n = 2$), v.r.g. ($n = 9$) and expiratory neurones in r.f.n. ($n = 5$) were tested for responsiveness to stimulation of r. obscurus and r. pallidus. Discharges of all of these neurones were depressed by stimulation (3–100 Hz) of r. obscurus at intensities (40–160 μA) which produced concomitant depression of phrenic nerve inspiratory discharges. Stimulation of r. pallidus, on the other hand, evoked firing from inspiratory and expiratory neurones in the medulla.

In Fig. 8, the responses of a d.r.g. inspiratory neurone to 3 Hz stimulation of r. pallidus are illustrated. Variability in the latencies of superimposed responses is evident. The neurone failed to follow all stimuli at frequencies greater than 30 Hz.

Therefore the responses, which could be evoked during expiration as well as inspiration, appear to be synaptic rather than antidromic. Firing to r. pallidus stimulation was recorded from v.r.g. and r.f.n. expiratory neurones, which also failed to follow faithfully stimulus frequencies greater than 30 Hz. Responses of expiratory r.f.n. neurones were evoked during expiration but not during inspiration.

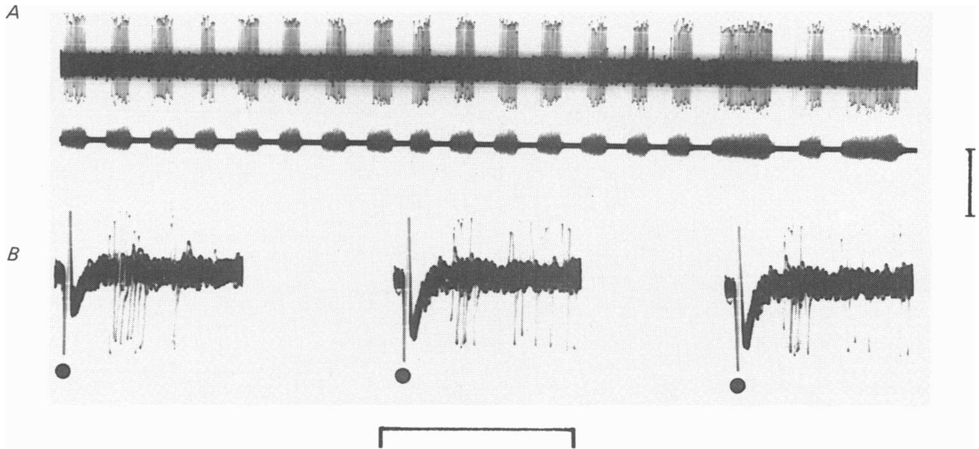

Fig. 8. Responses of d.r.g. neurone to stimulation of r. pallidus. *A*, film records of spontaneous discharges of d.r.g. neurone (top) and phrenic nerve (bottom). *B*, superimposed responses of d.r.g. neurone to 30 Hz stimulation of r. pallidus at intensity (60 μA) which produced firing on phrenic nerve similar to that shown in Fig. 1*B*. Voltage calibration is 500 μV. Time calibration is 12 s for *A*, 50 ms for *B*.

Effects of spinal lesions

The effects on phrenic nerve activity of lesions made in the ipsilateral spinal cord at C3 and C4 segments were investigated in four experiments. In each experiment, a series of cuts of increasing size was made with bent 30 gauge needles. Lesions which severed the ipsilateral dorsal column had no effect on spontaneous respiratory discharges or on the responses to raphe stimulation. Severing the ipsilateral dorsal and dorsolateral columns further caudally also had no effect. When larger lesions were made which severed the ventrolateral quadrant, spontaneous discharges were reduced markedly, however the stimulus–response relationship was similar to that observed before lesioning (Fig. 2). Excitatory responses to r. pallidus stimulation were also retained. This observation is noteworthy in light of recent evidence that bulbo-spinal axons of Botzinger expiratory neurones are found in the dorsal and medial parts of the lateral funiculus in the C4 and C5 segments of the spinal cord (Fedorko & Merrill, 1984). Lesioning of the ipsilateral ventrolateral and ventral quadrants on the other hand, eliminated all activity, including the response to r. pallidus stimulation.

Responses to raphe stimulation during activation of chemoreceptors

When cats were allowed to breathe spontaneously, escape from the characteristic responses to 100 Hz stimulation of r. obscurus, r. pallidus or r. magnus occurred (not illustrated). Escape from the apnoea produced by r. obscurus or r. magnus stimulation

or the apneusis during r. pallidus stimulation occurred within 10–20 s from the beginning of stimulation. The effect is attributed primarily to activation of peripheral and central nervous chemoreceptors by hypercapnia or hypoxia, since raphe stimulation was also much less effective in paralysed cats which were ventilated with 5% CO_2, or with 15% O_2 in N_2. The effects of hypercapnia on inhibition produced by r. obscurus stimulation are illustrated in Fig. 9. Rate-meter records of phrenic nerve

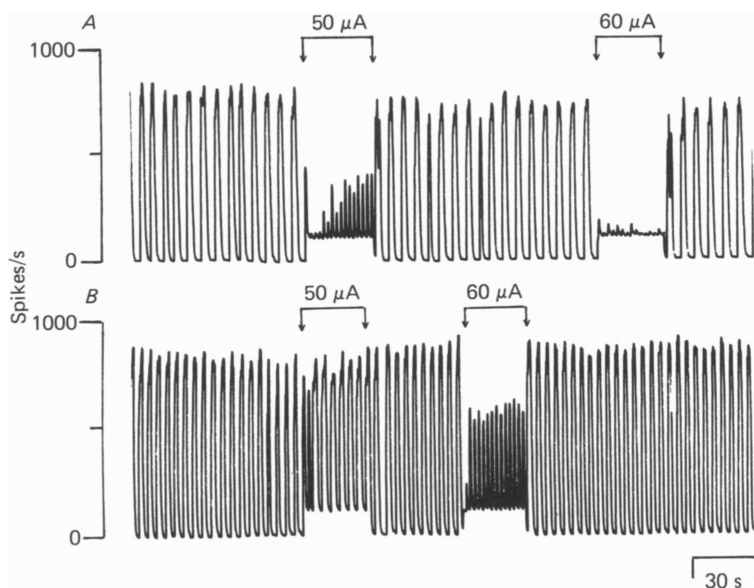

Fig. 9. Effects of hypercapnia on responses to stimulation of medullary raphe complex in artificially ventilated, chloralose–urethane-anaesthetized cat. *A*, rate-meter records of phrenic nerve activity during ventilation with 100% O_2. R. obscurus stimulated (100 Hz) with two current intensities (50 μA, 60 μA) during times denoted by arrows. *B*, records of phrenic nerve activity during ventilation with 5% CO_2–95% O_2. Sustained elevation of base line during raphe stimulation due to counting of stimulus artifacts.

activity recorded under control conditions are illustrated in the upper trace (*A*). The animal was ventilated artificially with 100% O_2 for 10 min prior to, and during, 100 Hz stimulation of r. obscurus. Responses to two intensities of stimulation are shown.

Responses in the lower record (*B*) were obtained during administration of 5% CO_2 in O_2. It can be seen that the inhibition of phrenic nerve activity during r. obscurus stimulation was attenuated greatly. The original effectiveness of raphe stimulation reappeared 3–5 min after cessation of CO_2 administration.

The tonic discharge produced by 100 Hz stimulation of r. pallidus was converted by hypercapnia to a pattern consisting of bursts of enhanced firing interrupted by pauses which are assumed to be expiratory related.

The effects of hypoxia were identical to those produced by hypercapnia. Typical responses to hypoxia are shown in Fig. 10. Control record *Aa* was obtained during ventilation with room air and with end-tidal CO_2 maintained at 4·2%. Under these conditions, 100 Hz stimulation of r. obscurus abolished spontaneous firing throughout

the period of stimulation. Responses in *Ab* were recorded during hypoxia, 20 s after beginning administration of 15 % O_2. Hypoxia increased spontaneous discharges and reduced the inhibitory effectiveness of r. obscurus stimulation. Control responses to r. pallidus stimulation are seen in *Ba*, with end-tidal CO_2 steady at 4·9 %. Responses in *Bb* were recorded 20 s after the start of ventilation with 15 % O_2. Similar to the effects of hypercapnia, an increase in peak inspiratory discharge frequency and a reduction in the tonic component of the response to pallidus stimulation are evident.

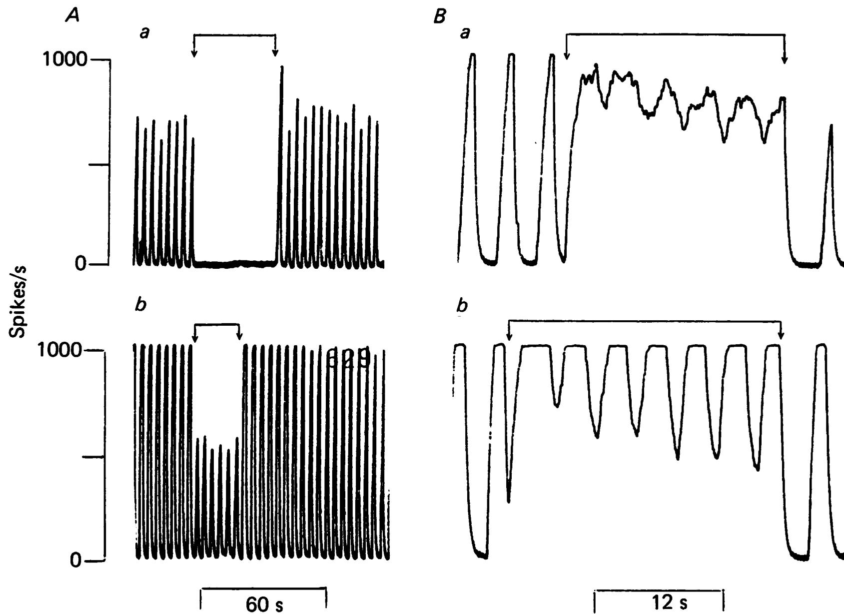

Fig. 10. Effects of hypoxia on responses to stimulation of medullary raphe complex in artificially ventilated, chloralose–urethane-anaesthetized cat. Upper records (*Aa* and *Ba*), rate-meter records of phrenic nerve activity during ventilation with 100 % O_2. *Aa*, stimulation (100 Hz) of r. obscurus; *Ba*, stimulation of r. pallidus. Note different time scales. Lower records (*Ab* and *Bb*), phrenic nerve activity during ventilation with 15 % O_2–85 % N_2. Stimulation of r. obscurus and r. pallidus in *Ab* and *Bb*, respectively, with stimulus parameters identical to those in *Aa* and *Ba*.

Responses during activation of baroreceptor and Hering–Breuer reflexes

Lung hyperinflation produced complete but transient inhibition of spontaneous phrenic nerve activity. Similarly, hypertensive episodes induced by metaraminol (30 μg/kg I.V.) abolished spontaneous firing. However, neither treatment in five experiments influenced the sustained discharge produced by r. pallidus stimulation, as evidenced by the essentially identical discharge patterns which occurred in the presence or absence of Hering–Breuer or baroreceptor reflex activation.

DISCUSSION

Comparisons between respiratory and cardiovascular responses to raphe stimulation

The region of the medulla now referred to as the caudal raphe complex was recognized years ago, on the basis of responses to electrical stimulation, as an area

involved in cardiovascular (Alexander, 1946) and respiratory (Pitts *et al.* 1939) regulation. Within recent years, more refined methods have been employed in an attempt to redefine the role of this region in respiratory and cardiovascular activities. Comparisons between previous studies and the present investigation do indeed reveal differences between the cardiovascular and respiratory-related responsiveness of the caudal raphe complex. In the present study, two patterns of respiratory reactivity were identified. Stimulation of points within a region encompassing most of r. obscurus and r. magnus resulted in depression, whereas stimulation of r. pallidus activated respiratory neurones. The over-all uniformity of respiratory neuronal responses evoked within each subdivision of the raphe complex differentiates them from cardiovascular responses, since pressor as well as depressor points are intermixed in all nuclei of the medullary raphe complex (Adair, Hamilton, Scappaticci, Helke & Gillis, 1977; Futuro-Neto & Coote, 1982; Yen, Blum & Spath, 1983; McCall, 1984). In addition, as shown in this study, stimulation of r. pallidus evokes firing of respiratory neurones, while other studies have shown that pallidus stimulation inhibits firing of thoracic preganglionic neurones and depresses spinal sympathetic reflexes (Coote & MacLeod, 1974, 1975). It would appear that respiratory neurones are more sensitive to raphe stimulation than sympathetic vasomotor neurones, since relatively modest depression of blood pressure occurs at intensities which evoke maximal changes in the firing of phrenic motoneurones. Consistent with this finding is the requirement for relatively high stimulus intensities to depress sympathetic reflexes by r. pallidus stimulation (Coote & MacLeod, 1974). It is also evident that the pathways mediating the respiratory and sympathetic neural responses to r. pallidus stimulation differ. Coote & MacLeod (1975) eliminated the depression of spinal sympathetic reflexes by sectioning the ipsilateral dorsolateral funiculus of the cervical spinal cord, whereas this procedure has no effect on the responses of phrenic motoneurones to r. pallidus stimulation. Finally, responses evoked in phrenic motoneurones occur at much shorter latencies than in sympathetic motoneurones, which receive input for the most part from small myelinated and non-myelinated bulbo-spinal axons. The short latencies of phrenic responses to r. pallidus stimulation could involve large myelinated raphe–spinal fibres, as well as myelinated and non-myelinated raphe fibres which synapse on medullary respiratory neurones. D.r.g. and v.r.g. bulbo-spinal axons are found in the ventral and ventrolateral spinal cord (Merrill, 1974), and based on the effects of spinal lesions seen in the present study, raphe–spinal projections to phrenic motoneurones would be expected to follow a similar route.

Results of the present investigation are in general agreement with two other studies dealing with respiratory effects of raphe stimulation. Pitts *et al.* (1939) recorded respiratory movements spirometrically during electrical stimulation of many different regions of the medulla, including the raphe nuclei. Although the study was carried out prior to the description and naming of raphe nuclei by Taber *et al.* (1961), regions corresponding to r. obscurus, r. pallidus and r. magnus are readily identifiable in Figs. 3 and 4 of their report. In agreement with the present results, inspiratory inhibition was produced by stimulating r. magnus and r. obscurus rostral to the obex, the greatest degree of inhibition being associated with the latter structure. Inspiration was augmented markedly during stimulation of r. pallidus,

whereas caudal to the obex, points producing inhibition and facilitation of firing were intermingled in r. obscurus. Sessle *et al.* (1981) examined the effect of stimulating raphe magnus on the discharge properties of respiratory neurones in the nucleus of the solitary tract. Weak to moderate inhibition of spontaneous inspiratory discharges were observed, whereas reflex responses related to swallowing, coughing and jaw movements were depressed greatly.

Neuronal substrates associated with respiratory responses

The many studies in which raphe stimulation has been used to evoke respiratory, cardiovascular and antinociceptive responses have assumed that the effects are predominantly due to stimulation of raphe neurones. The possibility of current spread to adjoining structures was entertained, and, in most instances, steps were taken to minimize it. In analysing the results of data presented here, the possibilities of current spread and activation of fibres of passage must be considered before concluding that raphe neurones are responsible for the altered respiratory discharge patterns.

Stimulus intensity, electrode configuration and the cytoarchitectonic features of the regions stimulated are determinants of the degree of current spread. In other studies, in which similar bipolar electrodes were used to stimulate raphe nuclei with an equivalent range of current strengths, it was estimated that current spreads a distance of 0·5–1·0 mm from the cathode (Ranck, 1975; Dostrovsky, Hu, Sessle & Sumino, 1982; Yen *et al.* 1983). The results of the present investigation are in agreement with those estimates. Stimulus–response relationships characteristic of r. obscurus (Fig. 2) did not change appreciably until the electrode approached the junctional region between r. pallidus and either of the other two medullary raphe nuclei (Fig. 7). Advancement of the electrode ventrally by an additional 0·5–1·0 mm, into r. pallidus, established the intensity–response relationship which was characteristic of r. pallidus. Thus, current spread was probably limited to 1 mm beyond the cathode. When the electrode was within r. obscurus, current must have spread laterally into the medial longitudinal fasciculus (m.l.f., Fig. 4), a region containing fibres from the medial vestibular nucleus which project to, and monosynaptically inhibit, cervical motoneurones (Wilson & Yoshida, 1969). It is unlikely, however, that m.l.f. axons are involved in the inhibition of phrenic motoneurones. Pitts *et al.* (1939) observed only a weak suppression of inspiration during stimulation of medial vestibular nucleus, and, in the present study, phrenic motoneurones did not appear to be inhibited monosynaptically. During stimulation of r. magnus or r. pallidus, current can spread to nucleus reticularis gigantocellularis (n.g.c.), therefore some of the effects on respiratory neurones may have been due to stimulation of n.g.c. neurones (Pitts *et al.* 1939).

Assuming that current is largely confined to the raphe nuclei, consideration must also be given to the identity and final destination of the neuronal elements stimulated. In addition to cell bodies, there are fibres, interspersed and arranged in bundles, which traverse the raphe in patterns characteristic of each nucleus (Taber, 1961). The electrodes used in the present experiments are capable of activating cell bodies as readily as myelinated and non-myelinated axons (Ranck, 1975; West & Wolstencroft, 1983); however, stimulation of decussating fibres passing through the

raphe from d.r.g., v.r.g. and r.f.n. does not appear to be responsible for the effects on phrenic motoneurones, at least within the usual range of stimulus intensities (40–160 μA) used in the present study. In particular, the inhibitions are not attributable to stimulation of Botzinger axons, which produce monosynaptic inhibition of d.r.g. neurones and phrenic motoneurones (Merrill *et al.* 1983; Merrill & Fedorko, 1984). Evidence against this possibility is provided by: (a) the concomitant increase in input resistance during inhibition of phrenic motoneurones, suggesting a mechanism of disfacilitation; (b) the maintained inhibition following sectioning of the dorsal and medial parts of the lateral funiculus in the cervical spinal cord, which would be expected to sever Botzinger bulbo-spinal axons (Fedorko & Merrill, 1984); (c) the absence of antidromic responses in Botzinger neurones during stimulation which inhibited phrenic motoneurones. In general, the maintenance of similar stimulus–response relationships, despite advancement of the stimulating electrode within any of the nuclei of the medullary raphe complex, is more indicative of activation of raphe cell bodies, since changes in threshold and reactivity would have been more likely associated with stimulation of individual fibres or fibre bundles. On the other hand, supramaximal stimulus intensities apparently activated d.r.g. and v.r.g. bulbo-spinal axons which produced the shorter latency responses seen in Fig. 1*D*.

Inhibition from r. obscurus or r. magnus and excitation via r. pallidus are exerted on medullary inspiratory neurones, since responses indicative of these influences were recorded from medullary neurones and from vagus nerve efferent fibres. However, effects at the spinal level, mediated by raphe bulbo-spinal neurones, cannot be totally excluded. Anatomical evidence exists for raphe projections to phrenic motoneurones (Dahlstrom & Fuxe, 1965; Holstege & Kuypers, 1982). Raphe-spinal axons travel in the ventrolateral and ventral funiculi, however the precise locations of the axon terminals have not been determined. It may be that raphe-spinal axons make axo-axonal connexions with d.r.g. bulbo-spinal axons. This anatomical arrangement would provide the means for presynaptic inhibition during raphe stimulation, and thus would explain the increased input resistance which accompanies the inhibition of phrenic motoneurones.

Characteristics of intracellular responses

The response to maximal stimulation of r. pallidus consists of sustained depolarization, decreased input resistance and increased discharge of action potentials. It is assumed that the synaptic mechanisms are qualitatively similar to those which generate the central respiratory drive potential in intercostal motoneurones (Sears, 1964) and phrenic motoneurones (Gill & Kuno, 1963; Biscoe & Sampson, 1970; Berger, 1979). Inhibition of spontaneous respiratory discharges during stimulation of r. magnus or r. obscurus by contrast was accompanied by a progressive increase in input resistance which eventually exceeded the resistances associated with the spontaneous expiratory phase (Figs. 5 and 6). The hyperpolarization of membrane potential, on the other hand, showed no further increase with duration of stimulation. No attempt was made to determine why further changes in membrane potential did not accompany the late increase in input resistance, however it is conceivable that reductions in both inhibitory and excitatory synaptic currents might have occurred.

Relative efficacies of inputs from raphe nuclei, from chemoreceptors and from cardiopulmonary afferents

Induction of hypercapnia or hypoxia in artificially ventilated cats produced alterations in responses to raphe stimulation that resembled those seen in spontaneously breathing cats. Inspiratory inhibition during r. magnus or r. obscurus stimulation was overcome during hypoxia or hypercapnia, whereas expiratory-phased inhibition was induced during r. pallidus stimulation by activation of chemoreceptors. Thus, evidence is presented for a common mechanism of chemoreceptor activation that excites expiratory as well as inspiratory neurones over more powerful or more strategically positioned synaptic inputs than those from the raphe complex. It is evident from other studies that peripheral and central nervous chemoreceptors activate very powerful and direct excitatory synaptic inputs onto phrenic motoneurones. Hypercapnia actually depresses phrenic motoneurones directly, as evidenced by an increased rheobase (Gill & Kuno, 1963), however, the depression is overcome by activation of excitatory bulbo-spinal pathways during chemoreceptor activation (Bainton *et al.* 1978). The depression of phrenic motoneurones by raphe stimulation is evidently not due to direct post-synaptic inhibition, but is related to disfacilitation. Thus, synaptic inputs from chemoreceptors apparently have more direct and powerful projections to phrenic motoneurones than those originating in the medullary raphe complex.

Phrenic nerve responses to r. pallidus stimulation are indicative of a predominant influence on the inspiratory pathway which increases with stimulus intensity until only small expiratory-phased pauses are evident. Hyperventilation also produces tonic firing of expiratory neurones (Batsel, 1967; Cohen, 1968; Bainton & Kirkwood, 1979), and r. pallidus stimulation evokes synaptic firing of expiratory as well as inspiratory medullary neurones. Therefore, it appears that inspiratory neurones receive a more direct or more powerful synaptic input from r. pallidus than expiratory neurones. A direct projection would also account for the maintenance of the response to pallidus stimulation during activation of baroreceptor or Hering–Breuer reflexes, which are known to depress medullary respiratory neurones (Gabriel & Seller, 1969; Cohen, 1969).

Functional implications

Pitts and co-workers (Pitts *et al.* 1939) concluded that the medullary reticular formation, including regions now recognized as the medullary raphe nuclei, is involved in generating the central respiratory pattern. However, since there are no reports of neurones discharging with a respiratory rhythmicity in the raphe nuclei or nearby reticular structures (Sears, 1966; Yen & Blum, 1984), it is doubtful that the medullary raphe complex is a primary component of the respiratory neural pattern generator. However, raphe neurones are activated by tactile, nociceptive, auditory and visual stimuli, as well as by baroreceptor activation (Yen & Blum, 1984). Furthermore, changes in cardiovascular, respiratory, autonomic and somatomotor reflexes are altered along with pain and tactile sensation by raphe stimulation. Therefore, it seems more likely that adaptations required for visceral and skeletal motor performance are co-ordinated within the medullary raphe nuclei.

The author wishes to thank Ruth P. Lalley and Susan S. Krey for assistance in preparation of the manuscript, and Drs D. Ballantyne, S. W. Mifflin and D. W. Richter (I. Institute of Physiology, University of Heidelberg) and Drs D. O. Smith and R. L. Moss (Department of Physiology, University of Wisconsin) for reading and improving the manuscript. Research supported by U.S.P.S.H. Grant HL 29563 and by funds from a U.S.P.H.S. General Research Support Grant to the University of Wisconsin Medical School.

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