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EXCITATORY AND INHIBITORY ACTIONS ON PHRENIC MOTONEURONES

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The preceding paper (Gill & Kuno, 1963) dealt with intrinsic properties of phrenic motoneurones which may determine their ability to discharge at high frequencies. The present study is concerned with extrinsic factors which control the excitability of phrenic motoneurones. It has been demonstrated that electrical stimulation of the medullary 'inspiratory centre' evokes a motor discharge in the phrenic nerve (Pitts, 1941, 1942, 1943; Purpura & Chatfield, 1953), while stimulation of the 'expiratory centre' reduces the inspiratory phrenic discharge (Pitts, 1941, 1942). The latter effect, however, has been attributed to a mutual inhibitory action between inspiratory and expiratory centres, implying that the central actions upon phrenic motoneurones are exclusively excitatory from the inspiratory centre. This suggestion was consistent with the finding that the slowly augmenting and abruptly subsiding type of inspiratory discharge pattern in phrenic motoneurones is essentially similar to that of the medullary inspiratory neurone (Gesell, Magee & Bricker, 1940; Hukuhara, Nakayama & Okada, 1954; Gill, 1963). Calma (1952) has found that volleys applied to a variety of somatic nerves cause excitation and inhibition of phrenic discharge. It was again envisaged that the effects of somatic afferent impulses are integrated at the medullary respiratory centre before being relayed to the phrenic motoneurones. However, integration may also occur at the level of phrenic motoneurones. The phrenic nerve contains afferent fibres (Hinsey, Hare & Phillips, 1939; Rijlant, 1942; Cardin, 1944; Cuénod, 1961; Yaşargil, 1962; Landau, Akert & Roberts, 1962), and stimulation of the central end of a phrenic root may cause transient inhibition of the inspiratory discharge in other roots of the ipsilateral and contralateral phrenic nerves (Rijlant, 1942). The relatively short latency of this effect suggests a segmental mechanism confined to the cervical cord. A recent study has also shown that thoracic respiratory motoneurones are the sites of convergence for excitatory and

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inhibitory pathways of central and segmental mechanisms (Eccles, Sears & Shealy, 1962). The present study provides new information on these aspects of the integrative function of phrenic motoneurones.

METHODS

The experiments were performed on cats under sodium pentobarbital anaesthesia (Nembutal; Abbott Laboratories). In a few experiments, spinal preparations were used, acutely decapitated under preliminary ether anaesthesia. All animals were paralysed by injection of gallamine triethiodide (Flaxedil; American Cyanamid Company), and maintained on artificial respiration. The dissection and experimental arrangements were generally similar to those described in the preceding paper (Gill & Kuno, 1963). The influences from the medullary respiratory centre upon phrenic motoneurones were usually avoided by overventilating the animal with pure oxygen. When needed, the respiratory centre was activated by switching the gas supply of the respirator from 100 % O_2 to 6 % CO_2 in O_2 (cf. Gill, 1963). Ventilation with CO_2 excess, besides evoking phrenic discharge with a respiratory periodicity, induced an increase in cardiac pulsation which often led to loss of already penetrated cells. Consequently, phrenic motoneurones were successfully studied only under moderate increase in the respiratory drive. Suprasegmental stimulation was carried out with monopolar tungsten or steel electrodes placed into the medulla or into the upper cervical cord. These electrodes had tip diameters of 20–100 μ. For medullary stimulation, the occipital bone was resected and the cerebellum was removed with a blunt spatula. Intracellular recording from phrenic motoneurones was performed with glass micropipettes filled with 2·0 M potassium citrate solution, except for a few experiments in which 2·7 M-KCl was used. Population responses of phrenic motoneurones were studied by the method described in the preceding paper (Gill & Kuno, 1963). Additional technical details are given in the appropriate sections of Results.

RESULTS

Inspiratory phrenic discharge

When the CO_2 concentration of the inhaled gas was raised following penetration with the micro-electrode, phrenic motoneurones often fired repetitively. The repetitive spike discharge arose from a slow depolarization which was concurrent with inspiratory discharges recorded from the central end of a severed phrenic nerve. Figure 1 shows an example of the potential changes obtained during two consecutive inspiratory periods. The frequency of the inspiratory discharge was low at the beginning and gradually increased to reach a maximum, then suddenly subsided. In some phrenic motoneurones, particularly those having a relatively high threshold for initiating spike discharges, the 'firing level' for a train of spikes remained unchanged throughout the discharge period. However, there was often a progressive increase in firing threshold, as shown in Fig. 1. In these cells a change in the firing level paralleled the degree of underlying slow depolarization. With decay of the slow depolarization at the end of an inspiratory phase, the firing threshold also decreased.

In about 40 % of phrenic motoneurones tested under similar conditions

cell firing was absent, despite the presence of a slow depolarization (Fig. 2*A*). When these cells were continuously depolarized by passing currents through the membrane, the cell firing started first only during the maximal level of the depolarization (Fig. 2*B*), then progressively spread over the whole depolarization period with an increase of the current intensity (Fig. 2*C* and *D*). The pattern of discharge frequency so obtained was similar to that shown in Fig. 1. Figure 3 shows plots of the reciprocal of discharge intervals of records shown in Fig. 1 (filled circles) and of Fig. 2*D* (open circles), together with a tracing of the slow depolarization shown in

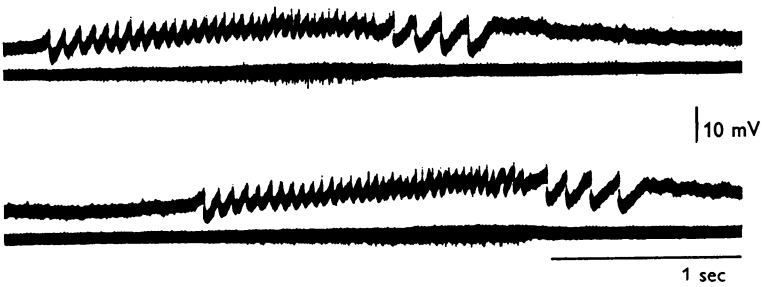

Fig. 1. Phrenic discharges during two consecutive inspiratory cycles. Upper beams, intracellular potentials from a phrenic motoneurone; upper part of spike potentials not visible in illustration; note slow depolarization. Lower beams, discharges recorded from the central end of a severed phrenic nerve trunk. Voltage scale for intracellular potentials.

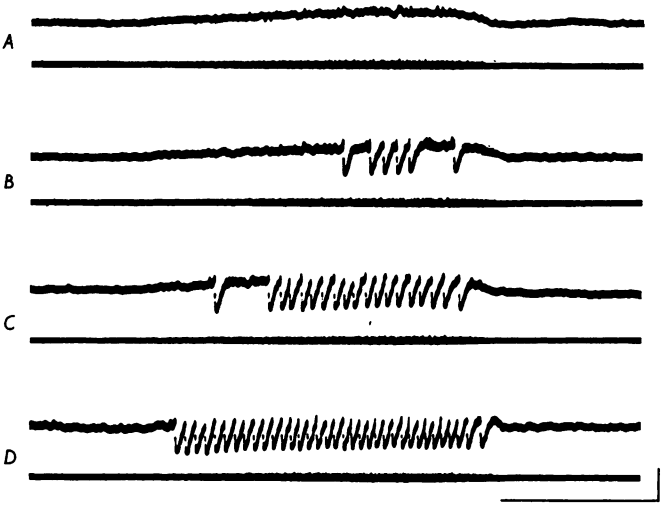

Fig. 2. Intracellular potentials from a phrenic motoneurone (upper beams) with discharges from phrenic nerve trunk (lower beams) during the inspiratory phase. Depolarizing current through the membrane was increased in intensity from *B* to *D*. Voltage, 10 mV for intracellular potentials. Time, 1 sec.

Fig. 2*A* on the same time scale. The timing of the successive discharges of such repetitive firing was closely correlated with the time course of underlying slow depolarization. This suggests that a major factor in determining the discharge pattern is the underlying slow depolarization; other factors such as accommodation do not seem to contribute significantly in limiting the duration and frequency of phrenic motoneurone discharges.

Figure 2*A* shows an apparent increase of synaptic 'noise' (small irregularly spaced potential waves) in phrenic motoneurones during the inspiratory phase. Although accurate observations have been made difficult by the dense distribution, some synaptic noise was depolarizing and some hyperpolarizing in phase. Further, diphasic or triphasic noise was often

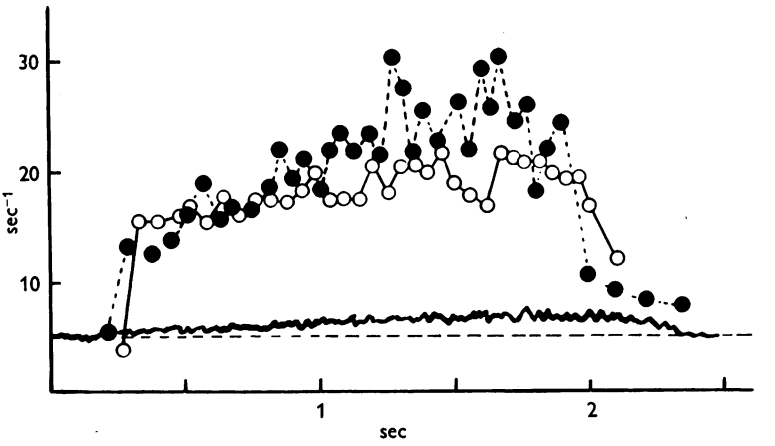

Fig. 3. Plots of reciprocal of interval between discharges of Fig. 1 (●) and of Fig. 2*D* (○) with a trace of slow depolarization of Fig. 2*A*. Abscissa, time after onset of slow depolarizations. Ordinate, frequency per sec.

recognized. The noise was more prominent near the maximal level of slow depolarization than at the beginning of the inspiratory phase. Detectable synaptic noise has never been observed during the expiratory phase. These observations suggest that periodic phrenic discharges are controlled by synaptic bombardment occurring only during the inspiratory phase.

Suprasegmental effects

Stimulation of descending tracts. Stimulation of the descending tract was carried out in spinal preparations transected at the atlanto-occipital membrane. Stimulating electrodes were placed in the lateral column of the cervical cord between segments C2/C3. Stimulation of this point invariably produced motor discharges in the phrenic nerve, although the responses sometimes disappeared during the experiment. No readjust-

ment of the position of the stimulating electrodes was made throughout the experiment. Typical membrane potential changes in phrenic motoneurones produced by stimulation of the descending tract are illustrated in Fig. 4*A* and *B*. The synaptic potential was usually complex, an initial depolarization being followed by a late hyperpolarization which was not separable by changing stimulus intensity. As seen below, the initial depolarization has been identified as an excitatory post-synaptic potential (EPSP) and the following hyperpolarization as an inhibitory post-synaptic

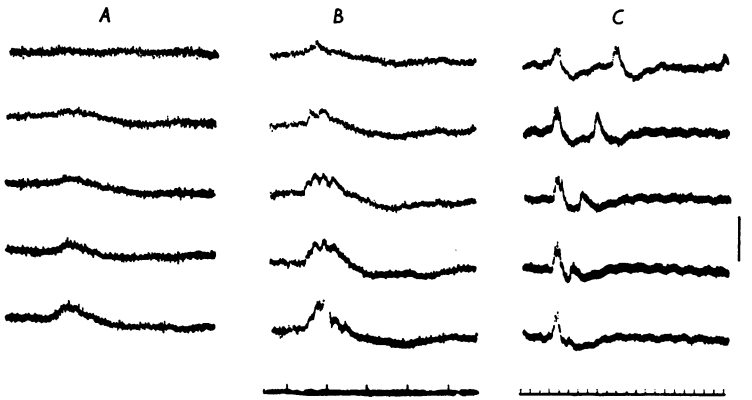

Fig. 4. Synaptic potentials in phrenic motoneurones evoked by stimulation of descending tract. *A*, stimulus intensity gradually increased from the top downwards. *B*, same as in *A* but recording from a different unit. *C*, same unit as in *B*. Two shocks applied at varying intervals. Voltage, 10 mV. Time, 10 msec. *A*, *B*, same sweep velocity.

potential (IPSP). The latency of the initial EPSP varied from 3·8 to 6·1 msec in different units, and the onset to peak time was approximately 5 msec. Since this EPSP gradually merged into a late IPSP, the duration of EPSP and the latency of IPSP were difficult to measure. The size and duration of the initial EPSP were apparently influenced by the size of late IPSP. For example, duration of an EPSP which had previously been about 8 msec was prolonged to approximately 23 msec when the late IPSP was spontaneously reduced to about one third of the initial size during impalement with a KCl micro-electrode (cf. Coombs, Eccles & Fatt, 1955). Although the duration of EPSP varied from 7 to 25 msec, depending upon the size of the late IPSP, it may be assumed that the EPSP would last for about 30 msec were it not contaminated by the late IPSP. The duration of IPSP was definitely longer than EPSP, being in the range of 50–120 msec. As shown in Fig. 4*A* and *B*, both EPSP and IPSP increased in size when the stimulus strength was intensified. At the bottom record of Fig. 4*B* a spike arose from the EPSP at a depolarization level of about 7 mV. It

should be noted, however, that spike generation did not appreciably change the time course of late IPSP, suggesting a large increase in membrane conductance during development of late IPSP. This assumption may also be supported by superposition of the EPSP and IPSP at various intervals, as illustrated in Fig. 4*C*. The size of EPSP produced by a second volley greatly diminished during the course of IPSP evoked by a first volley. This fact may indicate that the membrane conductance change during the IPSP is greater than that during the EPSP.

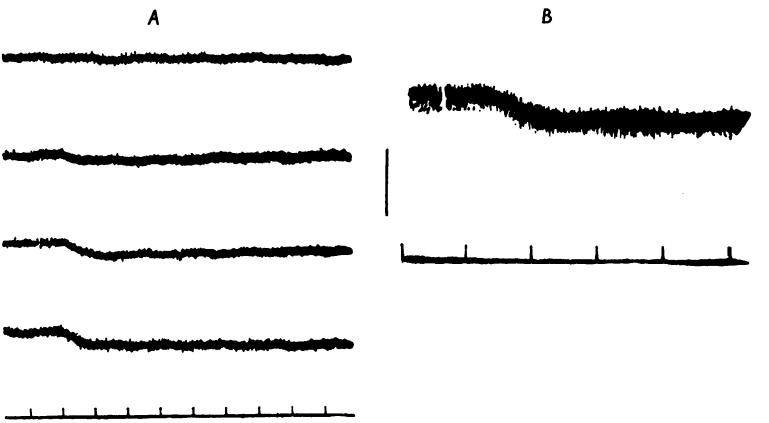

Fig. 5. IPSP in phrenic motoneurone evoked by stimulation of descending tract. *A*, stimulus intensity gradually increased from top to bottom. *B*, same unit but at higher amplification and faster sweep velocity. Voltage, 10 mV for *A* and 5 mV for *B*. Time, 10 msec.

Although a pure EPSP was never obtained by stimulation of the descending tract, pure IPSPs were observed in a few phrenic motoneurones. In the cell shown in Fig. 5 an increase in stimulus strength caused only a large IPSP without contamination from initial EPSP. The latency of the IPSP was found to be 7·1 msec (Fig. 5*B*), but this varied from 6·2 to 14·3 msec in different units. The duration of IPSP was approximately 100 msec, the range being 50–190 msec. These hyperpolarizing IPSPs could be changed to depolarizing responses by injection of Cl^- ions into the cell through the KCl micro-electrodes (Coombs *et al.* 1955).

Medullary stimulation. Stimulation of the medulla was performed only at two regions, both being at a depth of about 2 mm; one was located about 2 mm lateral and 1 mm cranial to the obex and the other was 3 mm lateral and 2 mm caudal to the obex. Haber, Kohn, Nagai, Holaday & Wang (1957) claim a greater likelihood of encountering more inspiratory cells and fewer expiratory cells in the former place and more expiratory with fewer inspiratory cells in the latter. Essentially similar

results have also been reported by von Baumgarten, von Baumgarten & Schaefer (1957). In this section the two places are respectively described as inspiratory and expiratory regions (see, however, Discussion).

Figure 6 illustrates the effects upon the response of a phrenic motoneurone population produced by conditioning volleys delivered to the inspiratory (*A*) and expiratory (*B*) regions. The test responses were

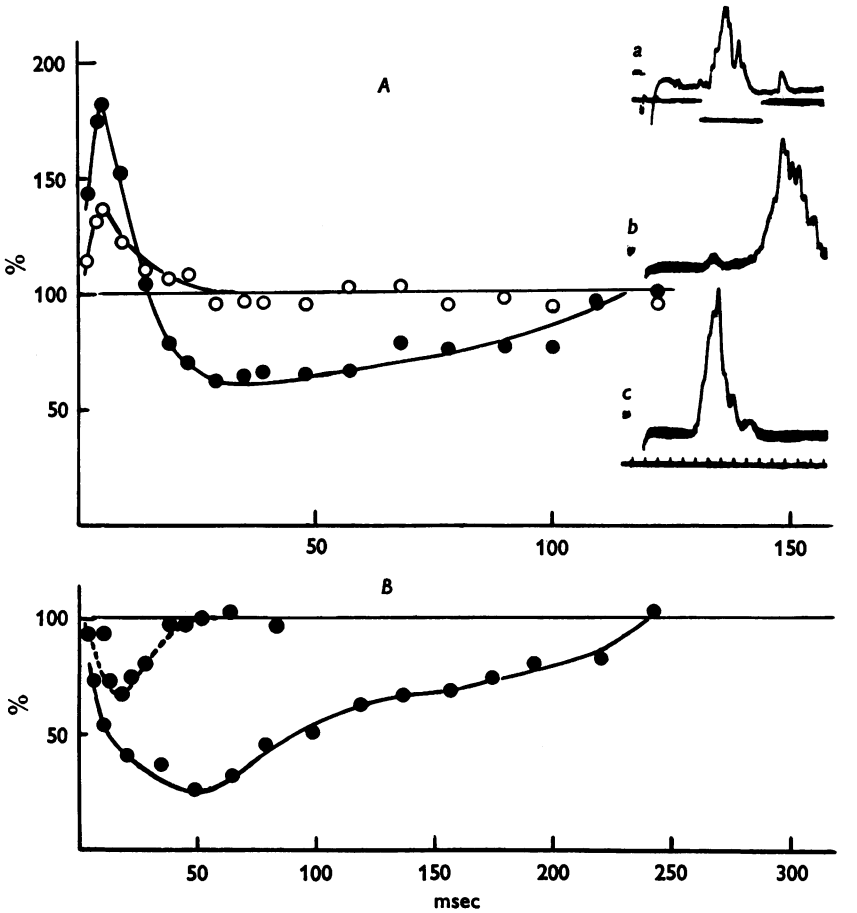

Fig. 6. Effects of medullary stimulation upon phrenic motoneurone population. *A*, stimulation of inspiratory region. ○, weak conditioning shocks (1·2 V, 0·1 msec pulse). ●, stronger conditioning shocks (2·4 V, 0·1 msec pulse). *B*, stimulation of expiratory centre in two different preparations. Ordinates, responses % of control. Abscissae, interval between conditioning and test shocks. Inset, *a*, response in the phrenic nerve following descending tract stimulation with a gate indicating width of response to be integrated; *b*, response in the phrenic nerve following stimulation of inspiratory centre; *c*, same as in *a*, but after spinal section at the atlanto-occipital membrane. Time, 1 msec.

evoked by stimulation of the descending tract in the upper cervical cord and measured by means of an electronic integrator (cf. Fig. 6*a*). In confirmation of Pitts (1943), phrenic responses were facilitated by weak conditioning shocks delivered to the inspiratory region (open circles), while this facilitation was followed by a prolonged depression when the conditioning stimuli were stronger (filled circles). On the other hand, when the conditioning shocks were given to the expiratory region, the phrenic response was invariably depressed irrespective of the conditioning stimulus intensity. The duration of depression, however, varied considerably in different preparations. Figure 6*B* gives the two extreme cases, one being about 50 msec, and the other approximately 250 msec in duration. In this series of experiments special care was taken to confirm that the test response used was not relayed to the medullary centre. As shown in the insets of Fig. 6 (*a*, *b* and *c*), the test response evoked by descending tract stimulation (*a*) had a shorter latency than the response produced by stimulation of the medullary inspiratory region (*b*). Further, the response following descending tract stimulation still remained after spinal transection at the atlanto-occipital membrane at the end of the experiments (Fig. 6*c*). Thus the sites of excitatory and inhibitory (or depressive) effects shown in Fig. 6 must have been located in the spinal cord.

Intracellular recordings of the responses following medullary stimulation were successfully made in eight phrenic motoneurones. Figure 7 illustrates the responses of two different phrenic motoneurones to stimulation of the inspiratory region. In contrast to the response evoked by descending tract stimulation (Fig. 4), the EPSPs shown in Fig. 7 were not significantly contaminated by the late IPSPs, although there was a slight prolonged hyperpolarization following the EPSP (cf. bottom record in Fig. 7*B*) which resembles a small undershoot of the monosynaptic EPSP in lumbosacral motoneurones (Brock, Coombs & Eccles, 1952). An increase in stimulus intensity from the top to the bottom in Fig. 7 showed an increase in amplitude of the EPSP without appreciable changes in duration. The peak time of the EPSP was approximately 5 msec, and the duration was 25–30 msec. These values are in good agreement with the time course of facilitatory action shown in Fig. 6*A* and of the initial EPSP evoked by descending tract stimulation.

Figure 8 shows two examples of prolonged hyperpolarizations produced by stimulation of the expiratory region. When the stimulus intensity was progressively increased from the top downwards, the responses obtained were usually pure IPSPs (Fig. 8*A*). In some cases hyperpolarization was preceded by a depolarization which occurred only during application of strong stimuli (Fig. 8*B*). This initial depolarization was apparently due to stimulus current spread over the cervical cord, since the latency was

much shorter than the EPSP produced by stimulation of the medullary inspiratory region. The total duration of the IPSPs was approximately 130–160 msec, which was in the range of the time course for inhibitory actions shown in Fig. 6*B* and for the IPSP evoked by descending tract stimulation.

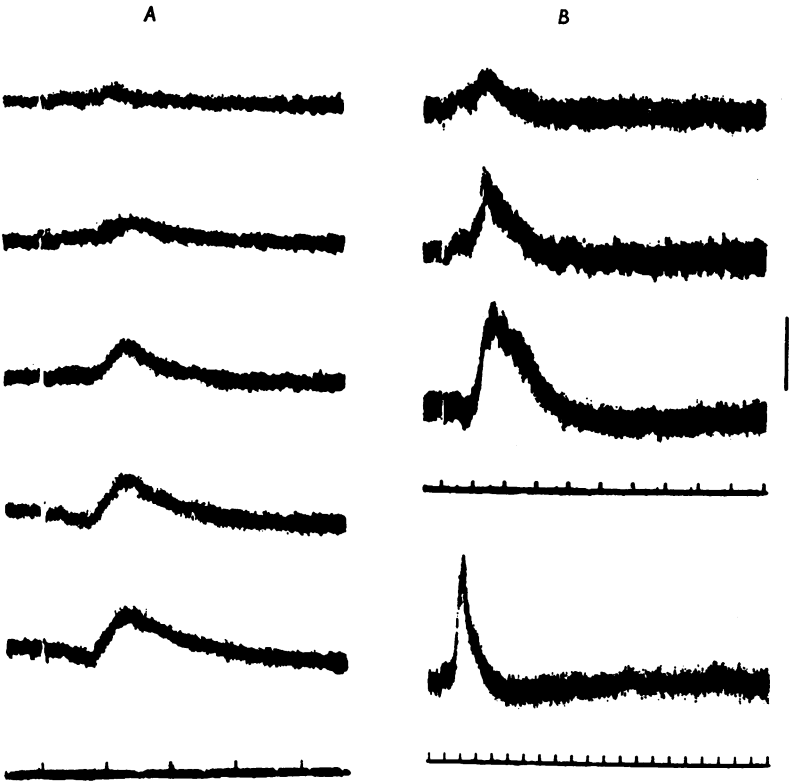

Fig. 7. EPSPs in phrenic motoneurones evoked by stimulation of inspiratory centre. *A*, *B*, different units. Stimulus intensities gradually increased from the top downwards. Voltage, 10 mV for *A* and 5 mV for *B*. Time, 10 msec.

For a single phrenic motoneurone the synaptic potentials evoked by medullary stimulation were altered by changing the depth of the stimulating electrode tip. In Fig. 9*A* the stimulating electrode placed in the inspiratory region was progressively advanced ventrally, stimulus intensity being unaltered. The amplitude of EPSP, which had previously initiated a spike potential (upper record), became smaller and a late IPSP appeared. A further advance of the stimulating electrode resulted in a disappearance of all the synaptic potentials (lower record). Similar observations are shown in Fig. 9*B*, in which a stimulating electrode

placed in the expiratory region was gradually moved ventrally. The synaptic potential was converted from IPSP to EPSP as shown from the top to the bottom. These findings may indicate that two separate systems which exert pure excitatory and inhibitory actions upon phrenic motoneurones are roughly segregated in the medulla but in part intermingled.

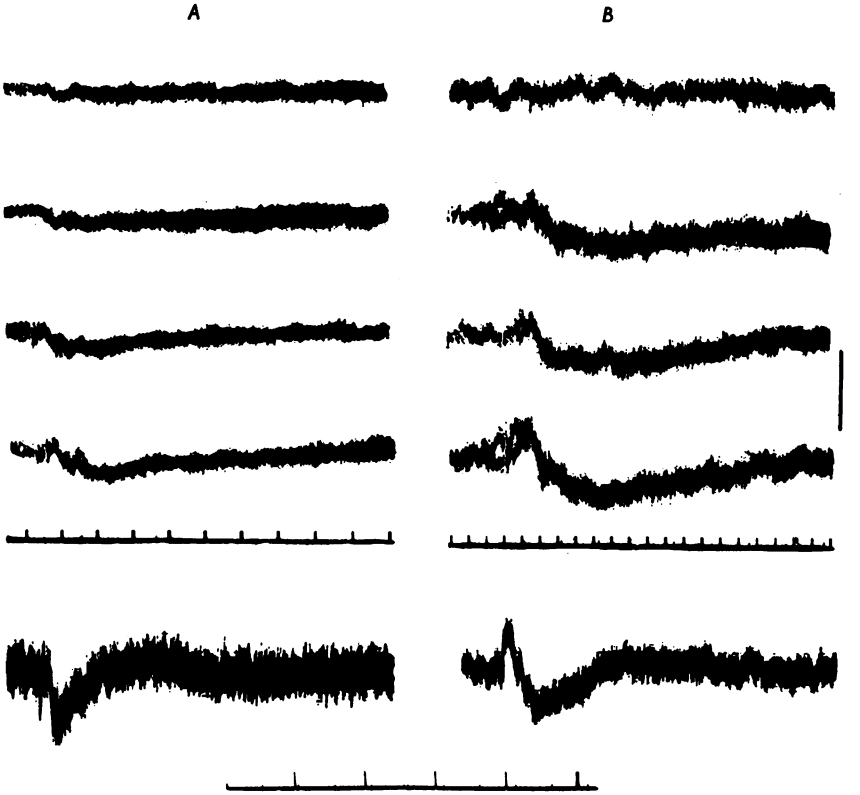

Fig. 8. IPSPs in two different phrenic motoneurones (*A*, *B*) evoked by stimulation of expiratory centre. Stimulus intensity progressively increased from the top downwards. Two bottom records, slower sweep velocity. Voltage, 10 mV for *A* and 5 mV for *B* and bottom of *A*. Time, 10 msec. Bottom time, 100 msec.

Segmental effects

Ipsilateral phrenic stimulation. Intracellular recording from phrenic motoneurones was carried out in spinal preparations in which all dorsal and ventral roots were left intact. In these preparations the responses evoked by stimulation of the ipsilateral phrenic nerve were only antidromic action potentials, and no synaptic potentials were detected. These experiments, however, were performed exclusively on one segment of the upper root of the phrenic nerve. Rijlant (1942) has shown that stimulation

of one root of the phrenic nerve inhibits the motor discharge in another root of the ipsilateral phrenic nerve. This effect was not tested in the present study.

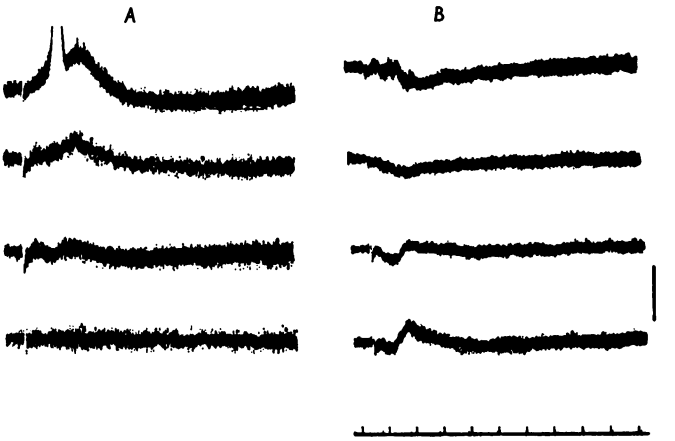

Fig. 9. Changes in synaptic potentials of phrenic motoneurones during vertical displacement of stimulating electrode placed in the medulla. Stimulating electrode gradually displaced ventrally from top to bottom records. *A*, stimulating electrode placed at about 2 mm lateral and 1 mm cranial to obex. *B*, stimulating electrode placed at about 3 mm lateral and 2 mm caudal to obex. Voltage, 5 mV. Time, 10 msec.

Contralateral phrenic stimulation. When the contralateral dorsal roots were left intact, stimulation of the upper root of the contralateral phrenic nerve produced a hyperpolarization in many phrenic motoneurones. This effect, however, showed considerable variation. The examples illustrated in Fig. 10 were obtained from three different units (*a*, *b* in *A*, and *B*). The latency of hyperpolarization was 5·8–9·1 msec, with a mean value of approximately 7 msec. The hyperpolarization usually lasted for about 15–20 msec. In a few cells, however, the duration was more than 40 msec (Fig. 10*Aa*). Sometimes measurement of the total duration was made difficult by occasional depolarization which followed the hyperpolarization (Fig. 10*Ab*). As shown in Fig. 10*B* this hyperpolarizing potential was similar to the IPSP of lumbosacral motoneurones, in that a depolarizing current passed through the cell increases the size of the hyperpolarization (middle trace), and a hyperpolarizing current (lower trace) converts the response into a depolarization (cf. Coombs *et al.* 1955). The effect from the contralateral phrenic nerve was observed in approximately 40% of phrenic motoneurones tested. In the rest of the units no potential change was detected.

In three cats the effects of contralateral phrenic volleys were tested while

recording population responses from the phrenic nerve following test stimulation of the descending tract in the upper cervical cord. Conditioning shocks were delivered to the central end of the upper root of the contralateral phrenic nerve which was cut distally. Figure 11 shows changes in the phrenic response relative to control size against the interval between conditioning and test stimuli. The time course of this inhibition in general corresponds to that of IPSPs presented in Fig. 10. Open and filled circles in Fig. 11 were obtained respectively before and after spinal section at the

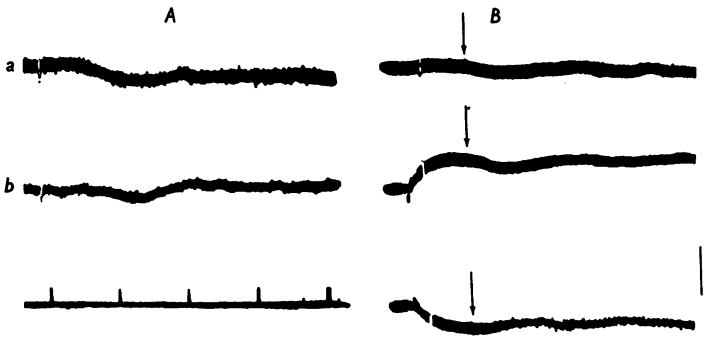

Fig. 10. IPSPs in phrenic motoneurones evoked by stimulation of contralateral phrenic nerve in preparations with intact dorsal roots. *Aa*, *Ab* and *B*, three different units. *B*: above, without polarizing current; middle, with depolarizing current; below, with hyperpolarizing current. Arrows indicate onset of IPSP. Voltage, 10 mV. Time, 10 msec.

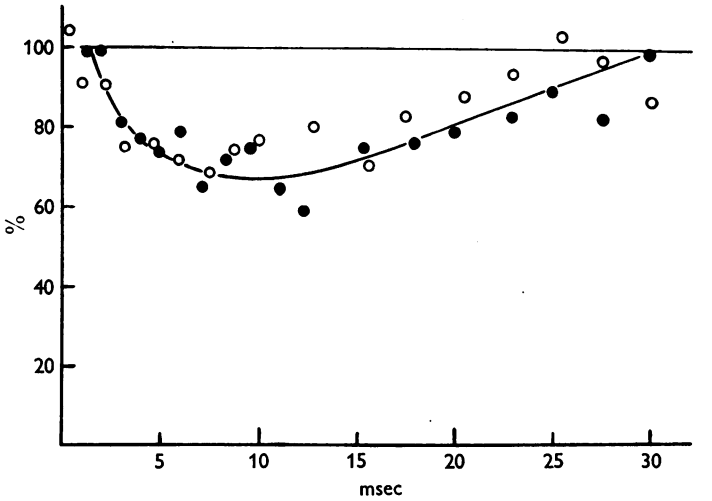

Fig. 11. Effect of stimulation of contralateral phrenic nerve upon phrenic motoneurone population. Ordinate, amplitude of response (% of control). Abscissa, interval between conditioning and test shocks. Before (○) and after (●) spinal section at the atlanto-occipital membrane.

atlanto-occipital membrane. Little difference in the inhibitory action before and after spinal transection indicates that this is a segmental effect in the cervical cord. The inhibitory effect increased gradually when the conditioning shocks were progressively intensified from 0·3 to 0·8 V with pulse duration of 0·1 msec. No additional effect was obtained by further increase in intensity of conditioning shocks, even up to 15 V. The inhibitory effect was completely abolished when the contralateral dorsal roots were cut. Since these results were essentially identical in the three cats, variations in the effect (see above) probably depend upon differences between phrenic motoneurones but not between different preparations. In fact, even in the same preparation some phrenic motoneurones showed hyperpolarization to contralateral phrenic volleys while others had no potential changes.

DISCUSSION

The present study has shown that impulses descending through the cervical cord produce both excitatory and inhibitory actions upon phrenic motoneurones. Stimulation of descending tracts in the upper cervical cord evoked EPSPs in phrenic motoneurones. This EPSP was invariably followed by a prolonged IPSP which could not be separated by changing stimulus intensity. However, since pure IPSPs of a similar time course have occasionally been obtained in a few phrenic motoneurones, it is assumed that the compound synaptic potential is derived from stimulation of two different types of descending fibres. The origins of these two systems were examined by stimulation of different medullary regions. It was found that stimulation at two selected positions produced reciprocal effects upon phrenic motoneurone population; one region, which is cranial to the obex, produced facilitatory effects, and the other region, which is caudal to the obex, exerted inhibitory effects. These effects were concomitant with the development of either EPSP or IPSP of a similar time course in individual phrenic motoneurones. The time courses of EPSP and IPSP so obtained were also similar to those of the initial EPSP and late IPSP evoked by stimulation of the descending tract in the upper cervical cord. Anatomically the stimulus site in the medulla which was found to produce pure EPSP corresponds to the region where inspiratory neurones had been most prominent when recording with micro-electrodes, and the place which evoked pure IPSP corresponds to the region where expiratory neurones had been found to predominate (Haber *et al.* 1957; von Baumgarten *et al.* 1957). It may thus be assumed that phrenic motoneurones are excited by descending impulses from the medullary inspiratory region and inhibited by discharges from the expiratory area. It could be argued that this may not be conclusive, since the anatomical localization of medullary

inspiratory and expiratory centres has not been well established (cf. Pitts, 1946; Hukuhara *et al.* 1954; Liljestrand, 1958; Salmoiraghi & Burns, 1960). However, the above assumption is not inconsistent with the findings that inspiratory and expiratory centres are to some extent intermingled in the medulla. In fact, even in the same level of the medulla, evoked post-synaptic potentials in phrenic motoneurones changed from EPSP to IPSP or *vice versa* when the stimulating electrode tip was moved vertically. Difficulty in separating pure EPSP from the complicated synaptic potentials produced by descending tract stimulation may also be accounted for on the same basis. It has been stated that both inspiratory and expiratory fibres descend side by side through the anterior and anterolateral columns in the cervical cord (Pitts, 1940; Hukuhara *et al.* 1954).

Another important mechanism for control of the activity of phrenic motoneurones is inhibition produced by afferent impulses in the contralateral phrenic nerve. This inhibitory action involves mechanisms at the cervical segmental level only, since spinal transection at the atlanto-occipital membrane did not alter the effect. Rijlant (1942) found a similar segmental inhibition and suggested a monosynaptic inhibitory pathway. However, the latency of the IPSP recorded in the present study was too long (about 7 msec) to assume a direct inhibitory action. Histologically the diameter spectrum of phrenic afferent fibres shows bimodal distribution (Hinsey *et al.* 1939; Landau *et al.* 1962). Stimulus strength used in the present study suggests that afferent fibres responsible for this inhibition are only large fibres.

The preceding paper (Gill & Kuno, 1963) and present studies have thus revealed that there are at least five factors that control the respiratory activity of phrenic motoneurones: (1) depression by after-hyperpolarization following each discharge, (2) depression by CO_2 excess, (3) excitatory action from the medullary inspiratory centre, (4) inhibitory control from the expiratory centre, and (5) segmental inhibition from the contralateral phrenic nerve. In quiet breathing, however, a major factor determining the discharge pattern of phrenic motoneurones seems to be only the underlying slow depolarization during the inspiratory phase. There was no evidence that phrenic motoneurones receive synaptic bombardment in the expiratory phase during quiet respiration. This agrees with the widely held idea that in quiet breathing expiration is largely a passive process, and active participation of expiration can be seen only when an extreme respiratory drive is demanded (Pitts, 1946). While no observations were made under an extreme respiratory drive, because of technical difficulties (see Methods), it is suggested that significant inhibitory control of phrenic motoneurones from the expiratory centre is probably exerted in such a situation. It should be noted that recording of inspira-

tory discharges from phrenic motoneurones has been performed in preparations with bilateral phrenic section. Yaşargil (1962) has distinguished two subgroups of large afferent fibres in the phrenic nerve by the phase of discharge during inspiration and expiration. A further attempt is required to assign the segmental inhibition to the subgroups of these large afferent fibres.

SUMMARY

1. Synaptic actions on phrenic motoneurones of the cat have been studied by intracellular recording and observing the response of phrenic motoneurone population to test stimuli.

2. When the CO_2 level of the inspired gas was raised, periodic repetitive firing occurred in phrenic motoneurones. The spike discharge arose from a slow depolarization. In some neurones only the slow depolarization was present, without cell firing. The repetitive discharge pattern was closely correlated with the time course of the slow depolarization. Synaptic noise was found on the depolarization wave but not during the expiratory phase.

3. Stimulation of the descending tract produced an initial EPSP followed by a prolonged IPSP. In a few phrenic motoneurones a pure IPSP of a similar time course was found.

4. Stimulation of the medulla at about 1 mm cranial and 2 mm lateral to the obex facilitated the activity of phrenic motoneurone population, although with stronger stimulation this facilitation was followed by a prolonged depression. Intracellularly, a pure EPSP in phrenic motoneurones was found by stimulation of the same region.

5. Stimulation of the medulla at about 2 mm caudal and 3 mm lateral to the obex produced a prolonged inhibition of phrenic motoneurone population. This was confirmed by intracellular recording of IPSP of a similar time course.

6. In spinal preparations stimulation of the contralateral phrenic nerve inhibited phrenic motoneurones. This effect was abolished by cutting the contralateral dorsal roots.

7. It is concluded that phrenic motoneurones are excited by descending impulses from the inspiratory centre and inhibited by impulses from the expiratory centre.

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