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THE SLOW POTENTIALS OF THORACIC RESPIRATORY MOTONEURONES AND THEIR RELATION TO BREATHING

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The alternating contractions of inspiratory and expiratory muscles causing the movements of breathing result from the alternating, periodic, repetitive discharge of inspiratory and expiratory motoneurones. In fact, however, nothing is known of the immediate cause of these activities although much has been learnt about the supra-spinal mechanisms which influence them. The need is thus indicated for a study of the mechanism of respiratory motoneurone discharge and to this end the technique of intracellular recording from neurones in the central nervous system (Brock, Coombs & Eccles, 1952; Woodbury & Patton, 1952) lends itself admirably.

The present paper describes intracellular recordings from inspiratory and expiratory motoneurones of the thoracic spinal cord of spontaneously breathing, anaesthetized cats. A brief report of some of these results has been previously published (Eccles, Sears & Shealy, 1962). Intracellular recording from phrenic motoneurones under conditions of artificial respiration and in response to electrical stimulation within the medulla has recently been described by Gill & Kuno (1963).

METHODS

A detailed description of the dissections and method of mounting the preparations, and of the electrical stimulation and recording techniques employed, is given in the preceding paper (Sears, 1964*a*).

RESULTS

As described previously (Sears, 1964*b*), volleys propagated antidromically in motor fibres of the intercostal nerves evoked in motoneurones soma-dendritic (SD) spikes which served both to identify impaled cells as motoneurones and, by virtue of their extracellular field potentials,

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helped towards locating the motoneurones in the ventral horn. Furthermore, motoneurones could be identified as inspiratory or expiratory respectively, according to whether antidromic invasion resulted from stimulating the external or internal intercostal nerves.

Periodic antidromic invasion. As the micro-electrode was slowly thrust into the spinal cord the intercostal nerves were stimulated at 10 shocks/sec at about five times the threshold of the nerve so as to excite all the alpha

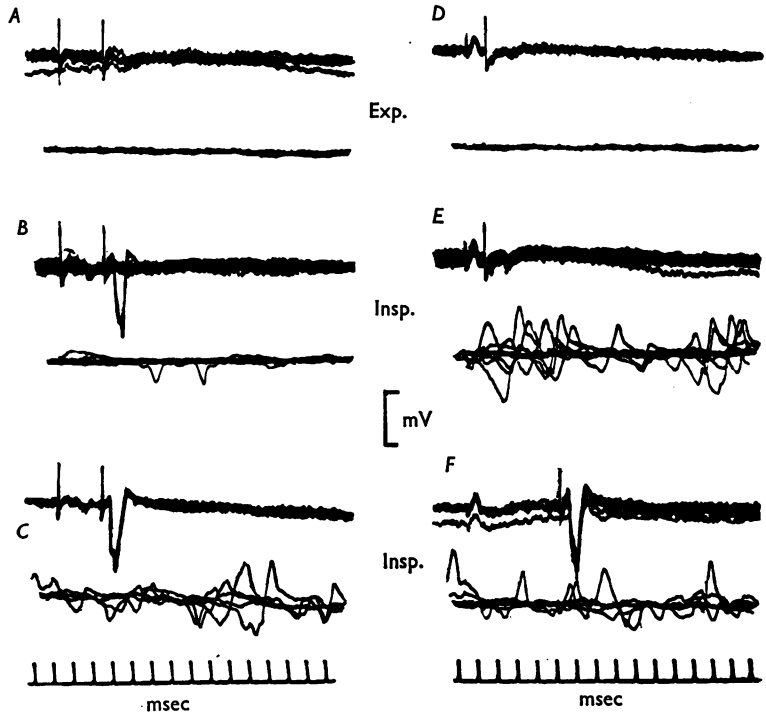

Fig. 1. Influence of CRDP on antidromic invasion. Upper traces, extracellular recording of SD field potentials (T9) evoked by stimulating the external intercostal nerve; lower traces, electromyogram of diaphragm. *A*, *B* and *C*, paired stimuli to the external intercostal nerve. *D*, *E* and *F*, stimulus to the external intercostal nerve preceded by stimulus to the corresponding internal intercostal nerve. The mV calibration refers to the upper traces.

motor fibres. In the spontaneously breathing animal it was frequently observed that when the micro-electrode was close to a motoneurone the antidromic SD spike occurred periodically in an 'all-or-nothing fashion', during inspiration in motoneurones activated by stimulating the external intercostal nerve; and during expiration for those excited antidromically after a stimulus to the internal intercostal nerve. For the inspiratory

motoneurone (T9) of Fig. 1, antidromic invasion occurred during inspiration but then only in occasional cycles. It never occurred during expiration even with the use of paired shocks to the external intercostal nerve in *A*, or with an antecedent shock to the internal intercostal nerve in *D*. However, when paired shocks were applied to the external intercostal nerve, antidromic SD spikes occurred in some sweeps when the stimuli were applied early during inspiration as in *B* (note minimal activity in diaphragm), and in all sweeps when the stimuli fell at the height of inspiration as in *C*. Similarly, in *F*, an antecedent shock to the internal intercostal nerve (during inspiration) facilitated antidromic SD invasion of the volley in the external intercostal nerve. Since antidromic SD invasion is aided by depolarization and opposed by hyperpolarization of the motoneurone membrane (Brock, Coombs & Eccles, 1953), such a result leads to the expectation that the membrane potential of the motoneurone, whose SD spike was recorded extracellularly, was subjected to some rhythmic modification. This motoneurone was not discharging spontaneously so the periodic antidromic invasion was not due to the collision of antidromic and orthodromic impulses. The purpose of using paired shocks was to sum the monosynaptic EPSPs thus evoked (Sears, 1964*b*) and so assist by depolarization the otherwise inconstant antidromic invasion.

Rhythmic fluctuations in membrane potential having a respiratory periodicity. During spontaneous respiration the membrane potentials of individual cells, to a varying degree, were subjected to slow rhythmic fluctuations having a respiratory periodicity as illustrated in Fig. 2. An analysis of the recordings made from the sixty-four expiratory motoneurones and twenty-three inspiratory motoneurones that were impaled, for periods of at least 3–4 min and up to 2 hr in the longest instance, revealed that these slow potentials showed certain constant characteristics which are enumerated under subheadings below.

(1) In motoneurones identified by antidromic invasion from the external intercostal nerve, the depolarizing phase of the slow potential was in phase with inspiration as denoted by the activity of the diaphragm (Fig. 2*A*, *B*, *C* and also Fig. 3; Fig. 4*B*; Fig. 7). Conversely, the depolarizing phase of the slow potentials recorded from motoneurones invaded antidromically from the internal intercostal nerve occurred during the expiratory pause (Fig. 2*D*, *E*, *F*; Figs. 4*A*; 6; 8). Thus in cells which according to independent criteria were identified as inspiratory or expiratory motoneurones, a depolarization occurred in the phase of the respiratory cycle when such a depolarization would be predicted. This relation was observed regularly in antidromically identified inspiratory and expiratory motoneurones both of the same animal (as in Fig. 4) and of different animals (Fig. 2).

(2) When periodic, repetitive firing of motoneurones occurred, the timing of the discharge coincided with the depolarizing phase of the slow potentials (Figs. 3, 4; see also Figs. 2, 3 of Eccles *et al.* 1962). Or, if the cell fired throughout the respiratory cycle, its discharge frequency was highest during the phase of depolarization (Fig. 5).

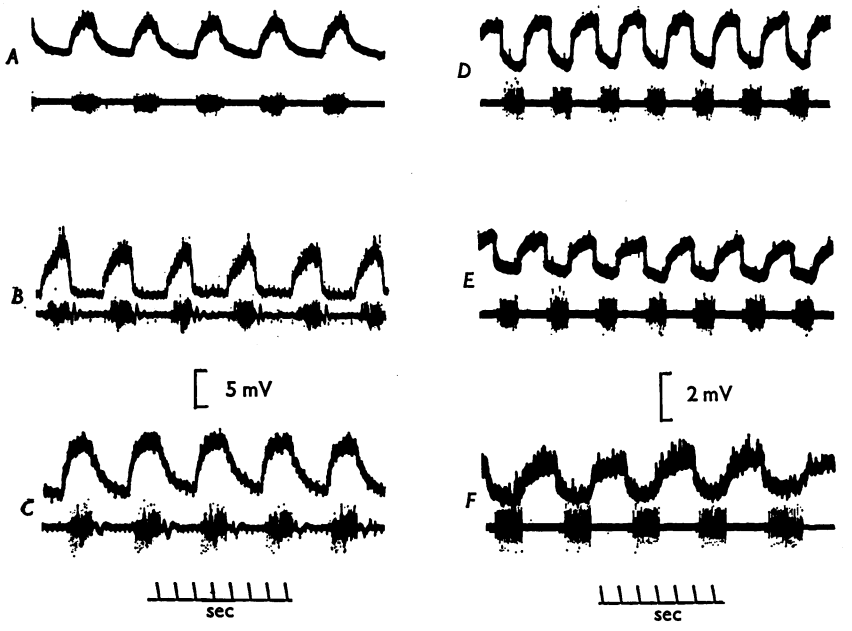

Fig. 2. Central respiratory drive potentials (CRDPs) of inspiratory and expiratory motoneurones, identified by antidromic invasion from the external and internal intercostal nerve respectively. Upper traces, intracellular d.c. recordings from inspiratory and expiratory motoneurones; lower traces, electromyogram of the diaphragm. *A*, *B*, *C*, inspiratory motoneurones; *D*, *E*, *F*, expiratory motoneurones. Note the difference in amplification for the records from the two types of motoneurone.

(3) In quiet breathing the maximum amplitudes of the slow potentials of the inspiratory motoneurones (range 5–12 mV) were greater than those of the expiratory motoneurones (0·5–6·0 mV) as illustrated in Fig. 2. This difference in amplitude of the slow potentials was associated with a corresponding difference in the amplitudes of the component waves of the 'synaptic noise' (Brock *et al.* 1952) superimposed on the slow potentials of the respective motoneurones. As a corollary, the impalement of an inspiratory motoneurone was invariably recognized over the loudspeaker before the test of antidromic invasion could be applied, by the characteristic intensity and rhythmic modulation of the 'synaptic noise'.

(4) Slow potentials of the form described were absent from recordings made from motoneurones in three animals in which the spinal cord was transected above the segmental level from which recording was made.

The above evidence supports the conclusion that the slow potentials are normal occurrences in thoracic motoneurones of the spontaneously breathing cat. Although it would be expected that any movements of the micro-electrode would be phase locked to the respiratory movements, the movements themselves could have any form. Furthermore, the movements could not consistently have given rise to potentials which were specifically phased in relation to the respiratory cycle yet occurred in functionally distinct motoneurones which had been identified by independent criteria. Movement artifacts could of course occur, especially when there were visible movements of the spinal cord. Such artifacts were usually recognized by their sharply contoured profile, the absence of related, periodic changes in the amplitude of the synaptic noise, and their non-systematic occurrence in inspiratory and expiratory motoneurones. Inconclusive but supporting evidence for the physiological basis of these potentials was obtained from recordings such as may be seen in Figs. 3, 5 (cf. Fig. 2 of Eccles *et al.* 1962), where the onset of the depolarization in the inspiratory motoneurone preceded the onset of discharge in the diaphragm, with which the greatest movement artifact would be expected to be linked.

Slow potentials were recorded from thoracic motoneurones subjected to a bilateral de-afferentation (acute) extending to three segments on either side of the segment from which recording was made. Since it has been established that thoracic motoneurones receive monosynaptic excitation principally from the same segment, with only a small contribution from the adjacent segment (Eccles *et al.* 1962), it is highly improbable that the slow potentials were dependent on proprioceptive reflexes from the chest and abdominal wall excited by the respiratory movements. Furthermore, the depolarizing phase of the slow potentials preceded such movements. It is therefore concluded that the slow potentials are 'central' in origin. In view of the causal relation between the slow potentials and the periodic discharge of respiratory motoneurones, it has been suggested that they be called 'central respiratory drive potentials' (abbreviated to CRDP).

A closer examination of the CRDPs is made possible by using the activity of the diaphragm electromyogram during inspiration as a reference phase. Shortly before the onset of diaphragmatic activity, the membrane potentials of inspiratory motoneurones usually show a small depolarizing step followed by a slower phase of depolarization which reaches a maximum in the latter third of inspiration. During this phase the base line thickens owing to a progressive increase in the amplitude of

the 'synaptic noise'. As inspiration proceeds, the 'synaptic noise' becomes progressively more rhythmic so causing the wavelets seen in many records, especially in motoneurones with high amplitude CRDPs (Figs. 2*A*, *B*, *C*; 4*B*; 5*C*, *D*; 6).

The repolarization of inspiratory motoneurones shows an initial rapid and a later slower phase which either continues progressively to increase throughout the entire expiratory pause (e.g. Figs. 2*A*, *C*; 4*B*) or reaches a plateau early in the pause and remains there until the next depolarization ensues (Fig. 4). The CRDPs of expiratory motoneurones were essentially of similar form except, of course, for their different phase relation to the activity in the diaphragm electromyogram. They could, however, differ in one important respect as may be seen in Fig. 4*A*. It will be noted that for the first two cycles of respiration the membrane potential remained virtually constant throughout the expiratory pause, whereas in the third and fourth cycles it showed a slight but definite progressive decrease during the pause. This progressive depolarization was most marked during the expiratory pause which followed removal of the vagal stimulation, the effects of which are later described. Other expiratory motoneurones showed an augmenting depolarization with each cycle. (Fig. 6*A*; see especially Fig. 3 of Eccles *et al.* 1962). On the other hand during *inspiration* the expiratory motoneurones invariably showed a progressively augmenting repolarization which did not reach a maximum until the latter half of inspiration (Figs. 2*D*, *F*; 4*A*; 5; 8). The significance of these different forms of the CRDPs is discussed later.

The excitatory phase of the central respiratory drive potential. As mentioned earlier, the repetitive discharges of respiratory motoneurones arise from the depolarizing phase of their CRDPs (Figs. 3; 5: Figs. 2 and 3 of Eccles *et al.* 1962). Because attention was focused on recording the CRDPs, few low-gain records were taken of the spikes. Also, at the low membrane potentials of many cells, the generation of spikes was very often suppressed by cathodal depression (cf. Coombs, Eccles & Fatt, 1955*b*) as shown by the return of spike activity when recording conditions spontaneously improved and the average membrane potential increased. It was difficult to determine the firing threshold under these conditions of orthodromic activation owing to the intense synaptic noise. Nevertheless, there was a small (1–2 mV) increase in spike threshold in motoneurones showing an augmenting frequency of discharge associated with an augmenting pattern of depolarization. In the motoneurones of Fig. 3 at 57 min and of Fig. 5*C*, firing threshold was reached only close to the level at which, atypically for inspiratory motoneurones, the CRDP showed a fairly long plateau of depolarization (cf. Fig. 2*A*, *B*, *C*). A consequence was that the discharge frequency was low (about 10 c/s) and the

spike amplitude was relatively constant (Fig. 5*E*) throughout inspiration. In contrast, Eccles *et al.* (1962) illustrated an inspiratory motoneurone the discharge of which accelerated from 8 to 20 c/s owing to an augmenting pattern of depolarization. In this cell the spike amplitude fell by as much as 2·0 mV at the height of the depolarization, this depression being associated with a corresponding reduction in the amplitude of the spike after-hyperpolarization. When, however, the motoneurone of Fig. 5 was

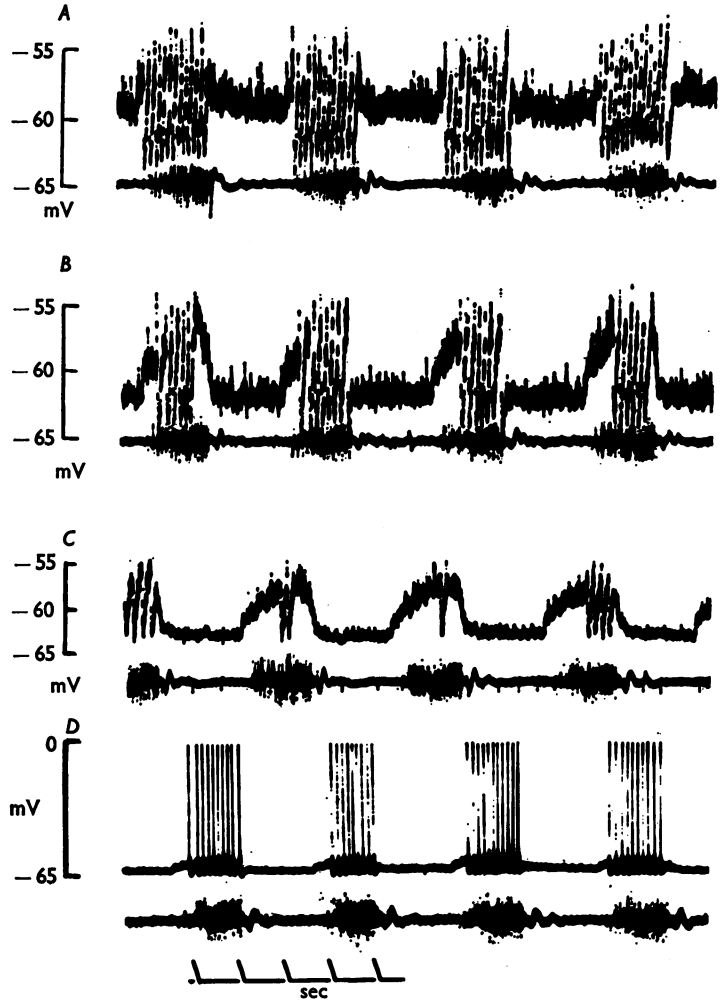

Fig. 3. Spontaneous changes in the discharge of an inspiratory motoneurone (T8). Upper traces, intracellular d.c. recordings: *A*, 29 min; *B*, 30 min; *C*, 42 min and, *D*, 57 min after impalement; note changes in amplification. Lower traces, electromyogram of diaphragm.

more depolarized as in *B*, so that it then showed an augmenting frequency of discharge before reaching a steady rate during the plateau, the amplitude of the after-hyperpolarization was diminished, particularly during the fifth cycle.

Although expiratory motoneurones were impaled with a higher incidence than inspiratory motoneurones (approximately 6:1) in 430 motoneurones from which records were actually taken, few showed repetitive firing, even though expiratory alpha motoneurone discharge may regularly be recorded from the internal intercostal muscles or the nerves which innervate them (Sears, 1963, 1964*a*). It has since been realized that a contributing factor was the certain reduction in alpha motoneurone excitability consequent to the near complete de-afferentiation of the segment which resulted from dividing the intercostal nerves for electrical stimulation (cf. effects of cutting the dorsal root on alpha motoneurone activity, Sears, 1964*a*). The greater chance of recording repetitive firing of inspiratory motoneurones was correlated with the greater amplitudes of their CRDPs.

During spontaneous respiration when stable recording conditions persisted for long periods (e.g. longer than 20 min) it was observed on several occasions that the average level of the membrane potential about which the CRDPs occurred slowly waxed and waned with a period ranging from 2 to 15 min. When the membrane potential was close to firing threshold the effect of these slow changes was very dramatic as shown for the series of recordings from an inspiratory motoneurone illustrated in Fig. 3. In the upper recording, made 29 min after impalement, the motoneurone discharged at a maximum rate of between 10 and 12 impulses per second during inspiration and the duration of the discharge closely paralleled that of the diaphragm activity. One minute later the level to which the membrane repolarized during expiration was 3–4 mV more negative than previously. The maximal discharge frequency was now lower at 8–10/sec and the duration of discharge much shorter because only the very summit of the depolarizing phase of the CRDP brought the membrane potential close to firing threshold. Twelve minutes later a further slight increase in the membrane potential (note gain change) resulted in a still lower frequency of discharge and the duration of discharge was so curtailed that in one cycle only a single discharge occurred. When, 15 min later, the membrane potential had decreased again, a regular sustained discharge occurred throughout almost the entire period that the diaphragm was active. This slowly occurring change in the pattern of discharge occurred repeatedly throughout the period of 110 min during which intracellular recording was made. The reason for believing that these recordings demonstrate a physiological regulation is that precisely similar patterns of alpha motoneurone discharge have been seen when recording efferent discharges

in intercostal nerve filaments. Furthermore, the discharge frequencies thus encountered fell within the same range as described above. These intracellular recordings of 'spontaneous' activity may therefore be distinguished from those made, for example, from the Betz cells of the motor cortex where the regular discharge obtained from them intracellularly could not be correlated with the pattern of activity recorded extracellularly before their impalement by the micro-electrode (Phillips, 1959).

Effects of electrical stimulation of the vagus nerve on the central respiratory drive potentials. As described in the previous paper low intensity repetitive stimulation (250–300 c/s) of the central end of the vagus nerve inhibits inspiration and prolongs the expiratory pause, during which time there may, or may not, be an active discharge of thoracic expiratory motoneurones

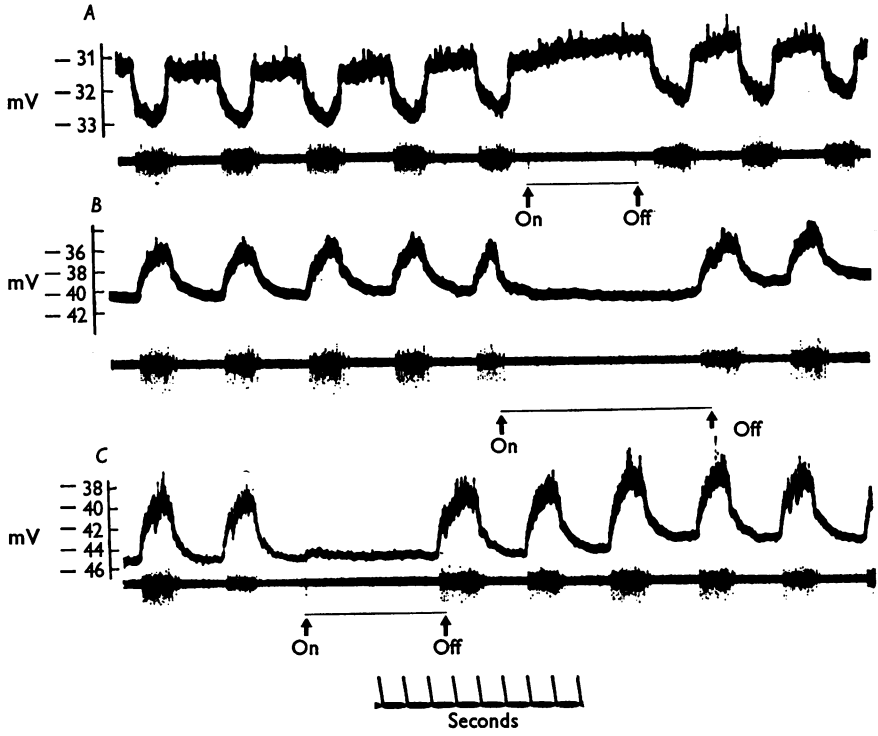

Fig. 4. Effects of stimulating the central end of the left vagus at 300 c/s on the CRDPs of inspiratory and expiratory motoneurones (T8). Upper traces, intracellular d.c. recordings from an expiratory motoneurone in *A*, and an inspiratory motoneurone in *B* and *C*; lower traces, electromyogram of diaphragm. *A* and *B* were recorded within 15 min of each other and their records have been aligned with respect to the diaphragm electromyogram. *C* was recorded 10 min after *B* when the membrane potential had increased.

(Sears, 1964*a*). During vagal stimulation the thoracic inspiratory motoneurones may 'escape' from the vagal restraint, and if stimulation is maintained each successive 'escape' increases in intensity. On the other hand, if the expiratory motoneurones become active during the expiratory pause, their discharge is inhibited with each 'escape'.

Intracellular recordings from inspiratory and expiratory motoneurones (T 8) during vagal stimulation are illustrated in Figs. 4, 5, 7 and 8. In Fig. 4 the recordings from an expiratory motoneurone (*A*) and an inspiratory motoneurone (*B*, *C*) were made within 15 min of each other. As the rhythm of breathing was unaltered during that time, the records have been aligned as if they had been recorded simultaneously. The onset of vagal stimulation fell at the beginning of the expiratory pause. Throughout the resulting prolonged expiratory pause there occurred a steadily increasing depolarization of the expiratory motoneurone. This depolarization was terminated when, after removing the vagal stimulus, the next inspiration occurred and the membrane repolarized, normal breathing then being resumed. For the inspiratory motoneurone, the onset of vagal stimulation fell in the middle of inspiration. This caused the abrupt termination of the depolarizing phase of the concurrent CRDP and the membrane repolarized to a level slightly higher than in any of the preceding expiratory pauses and remained there throughout the prolonged expiratory pause. Figure 4*C* shows a repeated trial of the effects of vagal stimulation. On this occasion the vagal stimulus fell just before inspiration and the expected depolarization was totally inhibited. During the prolonged expiratory pause the membrane potential remained at the same level, but there was a definite increase in the synaptic noise. In the post-stimulation period the amplitude of the CRDPs increased and the mean membrane potential shifted towards a more depolarized level.

The inhibition of inspiratory motoneurone discharge by vagal stimulation is illustrated in Fig. 5. In *A* the average level of the membrane potential was low so that the motoneurone discharged throughout the respiratory cycle, its discharge frequency increasing to about 15 impulses per second at the height of inspiration. In *B*, the membrane potential had increased and the discharge was now entirely periodic, the onset of inspiratory discharge now being seen to occur just after the onset of the depolarizing phase of the CRDP. Vagal stimulation in *B* interrupted the concurrent inspiration and the CRDP was so reduced in amplitude and duration that only three impulses were discharged before the prolonged expiratory pause ensued. Although stimulation was maintained, vagal 'escape' occurred twice, the second 'escape' being more powerful than the first, as judged by the higher initial rate of discharge before the vagal

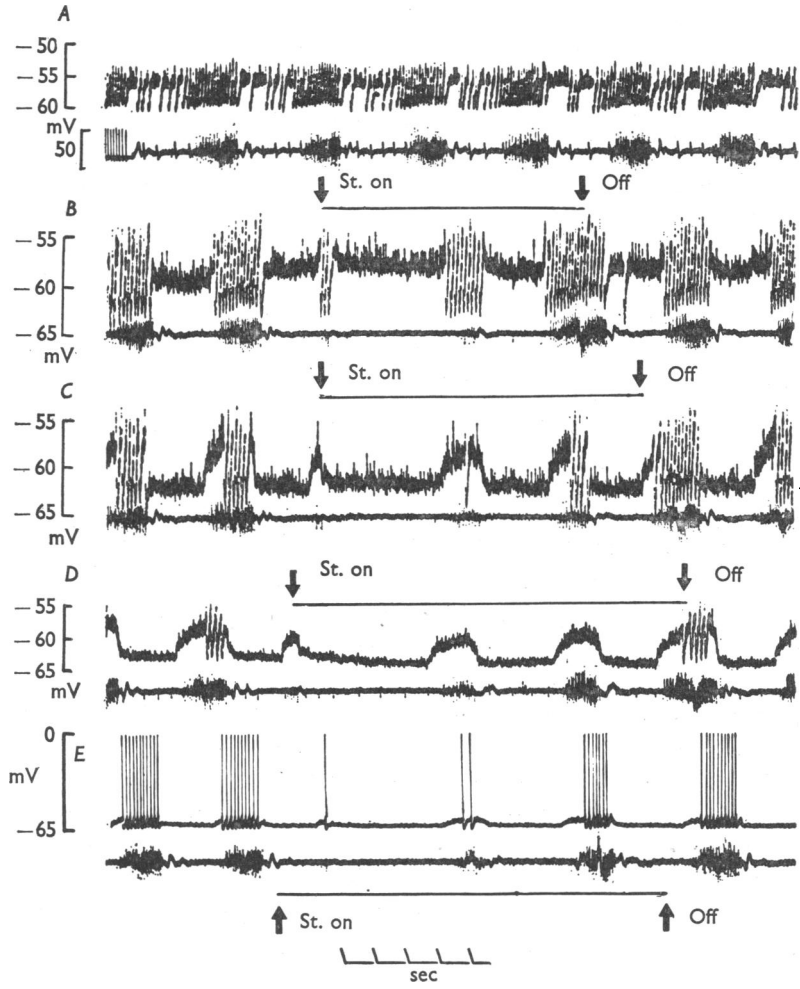

Fig. 5. Effects of electrical stimulation of the left vagus at 300 c/s on the discharge of an inspiratory motoneurone (same cell as in Fig. 3). Upper traces, intracellular d.c. recordings; note changes in amplification. *A*, *B*, *C*, *D* and *E* taken at 23, 29, 30, 42 and 57 min after penetration of the motoneurone. Period of vagal stimulation indicated between the arrows. Lower traces, electromyogram of the diaphragm.

stimulus was removed. In the control recording of *C*, the average membrane potential had further increased and the motoneurone only discharged near the summit of the CRDP. Repetition of the vagal stimulation at the same intensity and frequency again inhibited inspiration but although vagal 'escape' occurred in the diaphragm electromyogram the depolarization was now only adequate to discharge a single impulse in the

first 'escape' and four impulses in the second. In *D*, at a still higher average level of membrane potential, no discharge occurred with either the first or second 'escape'. During the prolonged expiratory pause in *D*, the membrane now repolarized to a slightly higher level than in the preceding expiratory pause. Figure 5*E* shows a recording at low gain of the inhibition of inspiratory motoneurone discharge by vagal stimulation.

The inhibitory phase of the central respiratory drive potential. Although the various concepts of the mode of operation of the 'respiratory centres' differ they have in common the view that inspiratory and expiratory motoneurones are subjected alternately to a periodic barrage of excitatory impulses from their respective centres. With respect to the maximal level of membrane polarization, the CRDP could be regarded as a monophasic wave of potential due simply to the waxing and waning of the postulated excitatory synaptic drives. The following evidence shows, however, that this explanation is not correct.

Some minutes after the impalement of respiratory motoneurones with KCl-filled micro-electrodes, it was often observed that the CRDP changes from a single to a double phase of depolarization per respiratory cycle. This change could occur without any appreciable change in membrane potential; and evoked activities, such as the antidromic SD spike or the monosynaptic EPSP, were unaffected. This kind of 'reversal' of the repolarizing phase of the CRDP occurred with KCl-filled electrodes, whereas it never occurred with electrodes filled with K-citrate (2M) in recordings made sequentially with the two types of electrode in the same or different animals (cf. Coombs Eccles & Fatt 1955*a*; Araki, Ito & Oscarsson, 1961; Ito, Kostyuk & Oshima, 1962).

According to the work of Coombs *et al.* (1955*a*), Araki *et al.* (1961), and Ito *et al.* (1962), 'reversal' of a repolarizing (hyperpolarizing) potential to a depolarizing potential would strongly suggest the presence of a phase of active inhibition of expiratory and inspiratory motoneurones during part at least of the repolarizing phases of their respective CRDPs. Their explanation of such a 'reversal' is that, owing to the diffusion of Cl^- ions from the micro-electrode, the Cl^- concentration within the cell is increased, so lowering the equilibrium potential for Cl^- ions. Hence during the specific increase in the K^+ and Cl^- ion conductances of the post-synaptic membrane (subsynaptic sites only) caused by the release of an inhibitory transmitter, the net flux of Cl^- ions is directed outwards, so tending to depolarize the membrane.

The following group of experiments were based on the assumption that similar events occurred in the respiratory motoneurones. Hyperpolarizing currents were passed through KCl-filled electrodes, so that Cl^- ions were injected into the respiratory motoneurones in order to accelerate the

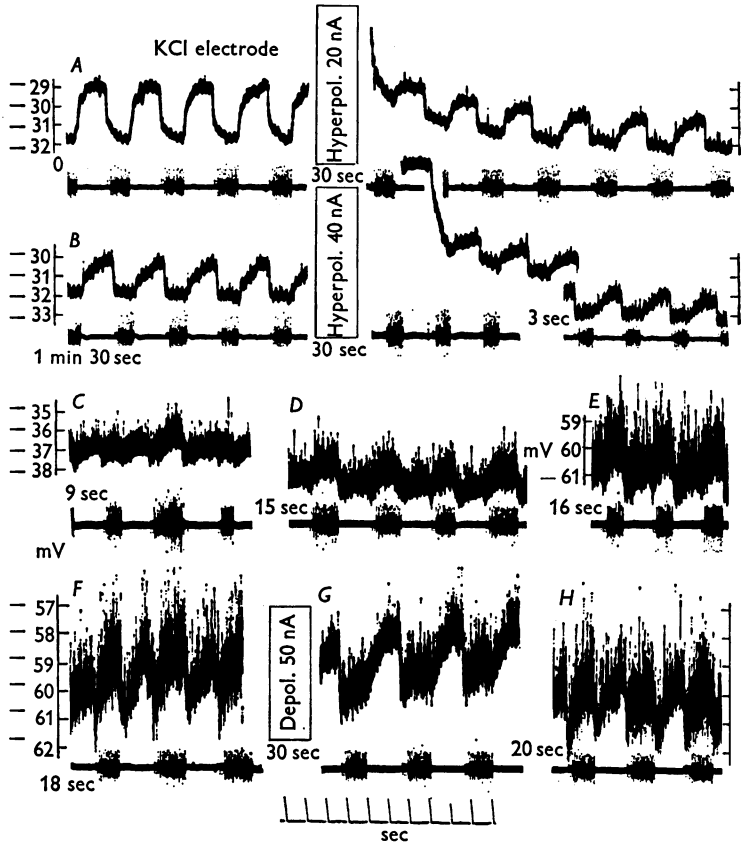

Fig. 6. Effects of passing hyperpolarizing and depolarizing currents through a KCl-filled micro-electrode on the CRDP of an expiratory motoneurone (T9). Upper traces, intracellular d.c. recording from an expiratory motoneurone (T9). *A* and *B* partially illustrate the effects of passing hyperpolarizing currents of 20 nA in *A* and 40 nA in *B* for 30 sec. *C*, *D*, *E* and *F* illustrate, at 9, 15, 16 and 18 min, the increase in membrane potential and synaptic noise which occurred spontaneously. Note change of gain in *E*, and the two phases of depolarization with each cycle of respiration due to the 'reversal' of inhibition. *G*, immediately after and *H*, approximately 1 min after depolarizing current of 50 nA for 30 sec.

otherwise slow and uncertain process of Cl^- ion diffusion. These experiments were difficult and frustrating. In addition to the problem of obtaining stable recording of the CRDPs, the prolonged passage of the ion-injecting current led most often to the loss of the cell, presumably by osmotically induced swelling. Injections were attempted in 50 cells and examples of the 'reversals' so caused are illustrated in Figs. 6 and 7.

When the expiratory motoneurone (T9) of Fig. 6 was first impaled the membrane potential was approximately -50 mV, but within a minute it had fallen to the level shown in the control record *A*. With each *inspiration* the membrane potential showed an augmenting repolarization and during each expiratory pause an augmenting depolarization. The injections of Cl^- ions were made by passing hyperpolarizing currents of 20 and 40×10^{-9}A for periods of 30 sec through the micro-electrode. By using the diaphragm electromyogram as the reference phase it may be seen that after the first injection the cell still showed an augmenting depolarization during expiration, whereas the augmenting repolarization during inspiration was almost abolished, especially when the membrane potential had returned to its former level. It should be noted that when the current was switched off the membrane potential fell immediately to below its former level (cf. Coombs *et al.* 1955*a*) and then rapidly recovered. The true time course of this recovery cannot be established since the effects of the current on the tip potential of this micro-electrode were not independently determined. After a considerable degree of recovery had occurred (note the repolarization during inspiration in the fourth cycle of *B*) a second injection was made. While the mean membrane potential was still low, immediately after cessation of the current, there was a small repolarization during inspiration (e.g. in the third inspiration). But 3 min later, when the membrane potential had increased, there was then a small but quite definite depolarization during *inspiration* (e.g. in the last inspiratory phase of the records in *B*). Thereafter, a steady increase in the average membrane potential occurred and this was accompanied by a striking increase in the synaptic noise (*C–H*) as previously observed following such injections by Araki *et al.* (1961). In *F*, there are two distinct waves of depolarization and repolarization in each respiratory cycle. Furthermore, the depolarization during inspiration is as large as that during the expiratory pause. When, in *C*, a more intense inspiratory discharge occurred in the diaphragm, there was an associated large increase in the depolarization. This association between inspiratory intensity and the magnitude of the hyperpolarization in expiratory motoneurones was observed regularly after 'reversal' had occurred.

The effect of depolarizing currents was then tried. According to Coombs *et al.* (1955*a*) a depolarizing current suppresses the anionic diffusion from the micro-electrode with the consequence that the diffusional exchange across the membrane allows the Cl^- ion concentration within the cell to approach its normal value.

The record in Fig. 6*G* was made immediately after removing a depolarizing current of 50×10^{-9}A through the micro-electrode for 30 sec. The effect of this was to abolish almost completely the depolarization during

inspiration without significantly affecting the depolarization during expiration. One minute later in *H* the record had returned to the same form as in *F*. The depolarizing current was applied three times in this motoneurone with exactly the same effect.

After 'reversal' due to a hyperpolarizing current had occurred, the record is seen to show a complete wave of depolarization and repolarization during expiration, followed by a complete wave of depolarization and repolarization during *inspiration*. This result is important and shows that a waxing and waning excitatory synaptic barrage (producing the depolarization during expiration) alternates with a waxing and waning inhibitory synaptic barrage (causing the depolarization during inspiration); some measure of temporal overlap of these two drives cannot be excluded.

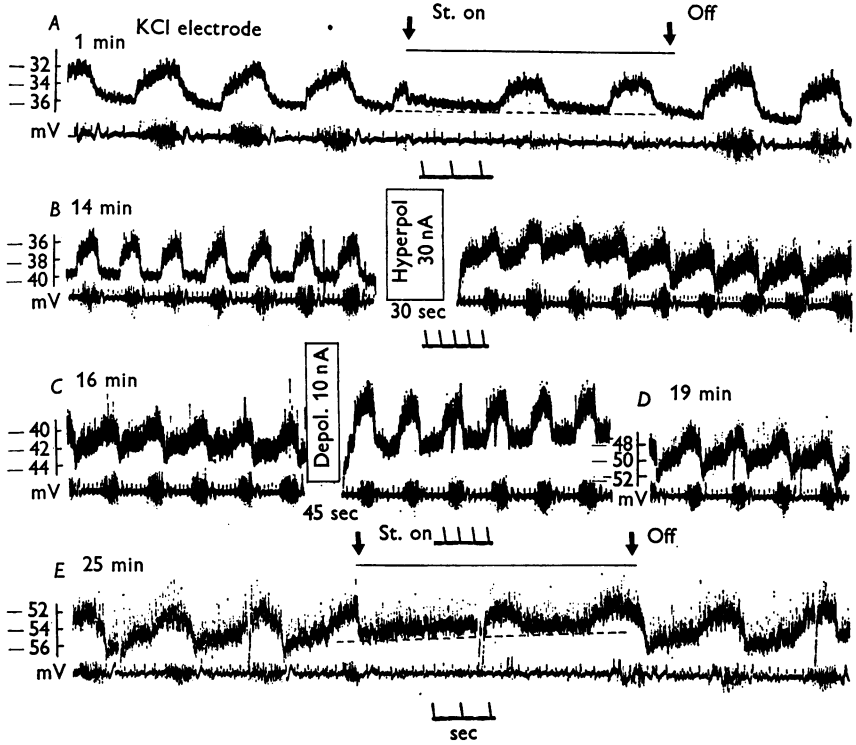

Fig. 7. Effects of passing hyperpolarizing and depolarizing currents through a KCl-filled electrode on the CRDP of an inspiratory motoneurone. Upper traces, intracellular d.c. recording (T8); lower traces, electromyogram of diaphragm. The control record *A* also shows the effect of stimulating the vagus nerve at 300 c/s for the period indicated by the bar line; *B*, effects of a hyperpolarizing current of 30 nA passed for 30 sec; *C*, effects of a depolarizing current of 10 nA passed for 45 sec; *E*, effects of vagal stimulation after 'reversal' of inhibition.

Similar 'reversals' were evoked by Cl^- ion injections into inspiratory motoneurones, as illustrated in Fig. 7. This figure also shows the effect of 'reversal' upon the responses evoked by vagal stimulation. In *A*, 1 min after impalement, the cell showed an augmenting depolarization during inspiration and a slight but progressive repolarization during the expiratory pause. Vagal stimulation inhibited inspiration and the depolarizing phase of the concurrent CRDP was abolished. The membrane polarization increased steadily throughout the prolonged expiratory pause as indicated by the interrupted line (Fig. 7*A*) until it was interrupted by two successive 'escapes'. In *B*, recorded 14 min later, the average membrane potential had increased and now the record showed no progressive repolarization during the expiratory pause, suggesting that reversal had already partially occurred owing to Cl^- ion diffusion from the micro-electrode. A hyperpolarizing current of 30×10^{-9}A was then passed. When the membrane potential had increased to its former average level, there were then two phases of depolarization per respiratory cycle. This 'reversal' was itself temporarily reversed by the application of a depolarizing current through the micro-electrode (*C*) but 2 min later the 'reversal' was re-established (*D*). After 'reversal' had occurred, stimulation of the vagus then caused a progressive depolarization throughout the prolonged expiratory pause (*E*, interrupted line) on which were superimposed the depolarizations due to each vagal 'escape'. After removing the vagal stimulation the membrane potential increased by 2–3 mV. This was due, first, to the completion of the concurrent inspiratory depolarization and, secondly, to the loss of the synaptic drive causing the reversed inhibition (depolarization) of the inspiratory motoneurone.

It is to be noted that, after 'reversal' has occurred, the form of the CRDP of an inspiratory motoneurone differs from that of an expiratory motoneurone in that the former shows more temporal overlap of the inhibitory and excitatory synaptic drives. Thus the depolarization during inspiration sums with the inhibitory depolarizing potential to form a humped wave of depolarization which occupies the entire respiratory cycle, the maximal repolarization of the membrane occurring at the end of inspiration. When vagal 'escape' occurs there is a corresponding repolarization of the expiratory motoneurones with each 'escape'. That this repolarization is in fact an inhibitory hyperpolarization is shown by the reversal which occurs when KCl-filled micro-electrodes are used or after the injection of Cl^- ions. In the control record of Fig. 8*A* the expiratory motoneurone (T9) showed a well-developed depolarization during the prolonged expiratory pause evoked by vagal stimulation. During the pause the membrane progressively depolarized until vagal 'escape' occurred, when it repolarized. After the 'escape' the motoneurone again

depolarized until vagal stimulation was terminated. Shortly afterwards in *B*, there was a spontaneous increase in membrane potential and this was associated with a reversal of the repolarization during inspiration to one of depolarization during inspiration. After 'reversal' had occurred the cell showed two phases of depolarization per respiratory cycle, the reversed inhibition being of greater amplitude than that of the expiratory

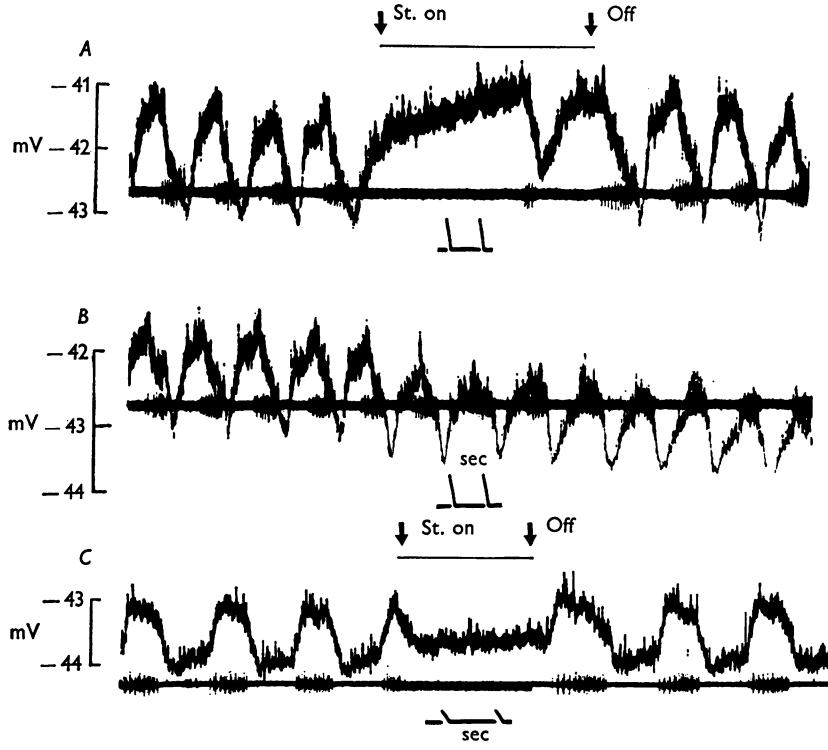

Fig. 8. Effect of vagal stimulation on the CRDP of an expiratory motoneurone (T9) before and after 'reversal' of inhibition. Upper traces, intracellular d.c. recording; lower traces, electromyogram of diaphragm. *A*, control; effects of stimulating central end of left vagus at 300 c/s. *B*, reversal assumed to be due to diffusion of Cl^- ions. *C*, effects of vagal stimulation after 'reversal'; note different time scales for *A* and *B*.

depolarization. That this interpretation is correct is shown by the fact that in *C* vagal stimulation abruptly shortened the concurrent, reversed inhibition. As described previously the magnitude of the inhibitory hyperpolarization is linked to the intensity of the inspiratory drive (Fig. 6*C*) so that when vagal inhibition caused a shortening of the concurrent inspiration the duration and magnitude of the inhibitory depolarization were similarly affected. Nevertheless, the expiratory motoneurone showed the

expected increasing depolarization throughout the prolonged expiratory pause (note different time scale for *C*).

DISCUSSION

During spontaneous respiration the thoracic respiratory motoneurones show rhythmic modifications of their membrane potentials which have been called 'central respiratory drive potentials' (Eccles *et al.* 1962), abbreviated to CRDP. On the basis of the experiments reported in the present paper the following interpretation is suggested. Each cycle of the CRDP is considered to comprise, first, a phase of depolarization and repolarization caused by a waxing and waning excitatory synaptic drive; secondly, a phase of hyperpolarization which follows, caused by a waxing and waning inhibitory synaptic drive. The periodic inhibitory and excitatory synaptic drives appear to follow each other in alternating sequence but there is asymmetry with regard to the extent to which these processes overlap. For both inspiratory and expiratory motoneurones the periodic excitatory synaptic drive probably wanes completely before the onset of the following periodic inhibitory drive. But in inspiratory motoneurones the periodic excitatory synaptic drive begins to build up before the inhibitory synaptic drive starts to wane as indicated by the single humped wave of depolarization for each cycle of respiration after reversal of the inhibitory post-synaptic potentials has occurred.

The conclusion reached above relates only to the *periodic* synaptic drives responsible for the CRDPs. The same excitatory and inhibitory pathways may be active throughout the respiratory cycle, a shifting balance between their respective intensities being responsible also for the average level of membrane potential about which the CRDP oscillates.

No information was obtained regarding the location of the cell bodies of the different neurones whose axons carry the impulses which evoke the CRDPs, that is to say, the *immediate* source of the CRDPs. Doubtless many systems of descending fibres from the brain stem could be implicated. Alternatively, the immediate source of the CRDPs may be spinal interneurones with short axons. The mechanism of respiratory motoneurone inhibition and excitation considered above must be distinguished from the over-all nervous mechanism of breathing whose activity gives rise to the excitatory and inhibitory synaptic drives producing the CRDPs in individual motoneurones.

The periodic active inhibition of thoracic respiratory motoneurones by hyperpolarization is as much a normal feature of their activity during spontaneous respiration as their periodic excitation by depolarization. Furthermore, the changes in respiratory motoneurone activity evoked by

vagal stimulation are due to modifications in the intensities and durations both of the inhibitory and of the excitatory synaptic drives. According to Gill & Kuno (1963) phrenic motoneurones receive synaptic bombardment only during the inspiratory phase in quiet respiration. It should be noted, however, that their animals were not breathing spontaneously.

The alternating inhibition of thoracic inspiratory and expiratory motoneurones during spontaneous respiration possibly serves to ensure that these motoneurones are not reflexly excited by the movements which occur when their antagonistic motoneurones discharge. Thus an increase in the intensity of the inspiratory discharge was associated with an increase in the concomitant hyperpolarization of the expiratory motoneurones. This mechanism acts conjointly with the recently described rhythmic modulation of intercostal fusimotor neurone discharge (Sears, 1962, 1963, 1964*a*; Critchlow & von Euler, 1962, 1963; Eklund, von Euler & Rutkowski, 1964) to produce a smooth regulation of the respiratory movement during spontaneous respiration.

The different forms of the CRDPs provide an explanation of the different patterns of discharge of alpha respiratory motoneurones recorded either electromyographically or from the nerves which innervate respiratory muscles (e.g. Adrian & Bronk, 1928; Bronk & Ferguson, 1934; Gesell, Magee & Bricker, 1940; Sears, 1963). In a following paper (in preparation) recordings are described from a thoracic motoneurone which show an approximately linear relation between membrane depolarization (synaptically induced) and discharge frequency with a slope of approximately 4·2 impulses per mV depolarization. Thus, within limits imposed by possible accommodation, the frequency of discharge will alter with a time course and magnitude similar to that of the CRDP, or at least to that part of it during which the membrane potential falls below firing threshold. This relation is clearly demonstrated in the records of Figs. 3 and 5, where the firing pattern depends both on the form of the CRDP and the average level of the membrane potential.

The depression of spike amplitude during orthodromic activation is of interest since previous studies on lumbo-sacral motoneurones (Brock *et al.* 1952; Coombs *et al.* 1955*b*) have failed to disclose the expected depression of the antidromic spike according to the short-circuit theory of EPSP production. On the other hand Fadiga & Brookhart (1960) have described such a depression of the antidromic spike of frog motoneurones during synaptic activation by fibres of the lateral funiculus. These considerations raise the possibility that in the mammal the segmental and central tracts may also differ in their site of termination on motoneurones but more quantitative studies are clearly required to settle this important issue.

In conclusion the present studies indicate that the respiratory motoneurone itself is of supreme importance as the final level of integration of the inhibitory and excitatory synaptic drives responsible for its periodic activity.

SUMMARY

1. A study has been made with intracellular recording of the synaptic actions exerted on respiratory motoneurones of the cat's thoracic spinal cord during spontaneous breathing.

2. Inspiratory and expiratory motoneurones are shown to be subject to slow, rhythmic changes in their membrane potentials which have been called 'central respiratory drive potentials' (CRDPs).

3. The CRDPs were analysed by examining the changes caused by the injection of Cl^- ions. It is suggested that the CRDPs are caused by alternating, waxing and waning, excitatory and inhibitory synaptic drives which respectively depolarize and hyperpolarize the motoneurone membrane.

4. The depolarization of inspiratory motoneurones occurs concomitantly with the hyperpolarization of expiratory motoneurones and vice versa. These two actions are largely interdependent. Thus increased inspiratory activity is associated with a greater inhibition of expiratory motoneurones.

5. The time course of the periodic discharge of respiratory motoneurones is determined by the depolarizing phase of their CRDPs.

6. High frequency, low-intensity stimulation of the central end of the vagus nerve inhibits inspiration and prolongs the expiratory pause, during which time the inspiratory motoneurones are actively inhibited by hyperpolarization and expiratory motoneurones are facilitated by depolarization.

7. It is suggested that the periodic hyperpolarization of inspiratory and expiratory motoneurones serves during spontaneous breathing to inhibit the proprioceptive reflex excitation that might otherwise occur consequent to the discharge of their respective antagonistic motoneurones.

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