ACTA NEUROBIOL. EXP. 1973, 33: 427-432
Lecture delivered at Symposium "Neural control of breathing"
held in Warszawa, August 1971

INVESTIGATION OF AFFERENT ACTIVITY IN THE INTACT PHRENIC NERVE WITH BIPOLAR ELECTRODES

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Abstract. The electrical activity of intact branches of the phrenic nerve was studied during spontaneous respiration in the anaesthetized or decerebrate cat. The action potentials were displayed with opposite polarity according to whether their activity was of afferent or efferent origin. The afferent volleys could be grouped according to three modalities. The first group had a clear silent period during diaphragmatic contraction (72%). The second group had volleys during inspiration (25%). The third group (3%) had rhythmic bursts of fast potentials that were independent of the respiratory cycle.

INTRODUCTION

Up to now, afferent impulses in the phrenic nerve have been recorded either at the level of the dorsal spinal roots C_4, C_5, C_6, (Corda et al. 1965) or at the peripheral end of the nerve itself or one of its cervical branches (Cuenod 1961, Glebovskii 1962). The nerve sections needed in these preparations might induce a disorganization of the motor function of the diaphragm as well as influence the afferent volleys. To eliminate this distortion during the present study the afferent impulses were recorded during spontaneous respiration with the phrenic pathway intact. Moreover further information about the nature and the origin of these impulses was obtained by different techniques which permitted the diaphragm tension to be varied.

MATERIAL AND METHODS

Twenty five adult cats of either sex weighing between 3 and 3.5 kg were used. Investigation of the electrical activity of the phrenic nerve during normal and spontaneous breathing was only made on the intact cervical branches of this nerve. Conditions of ventilation were such that the thoraco-diaphragmatic motor functions remained normal and there was restricted surgical destruction of the thorax and its innervation. The cervical branches of the phrenic nerve were easily reached at the level of their emergence from the scalene muscles. These anatomo-physiological factors and certain experimental conditions (e.g. the oil bath for immersion of the nerve and access to the diaphragm during the experiment) required the animal to be supine. The cats were either anaesthetized with pentobarbitone or decerebrated by transcollicular section of the brain stem.

In each experiment the animal was tracheotomized. The vagus nerves were isolated to be cut later. The abdomen was opened and the electrodes for recording electrical activity of the diaphragm were placed on the right and left sides of this muscle. Decerebration was performed under halothane anaesthesia before dissection of the phrenic nerve branches.

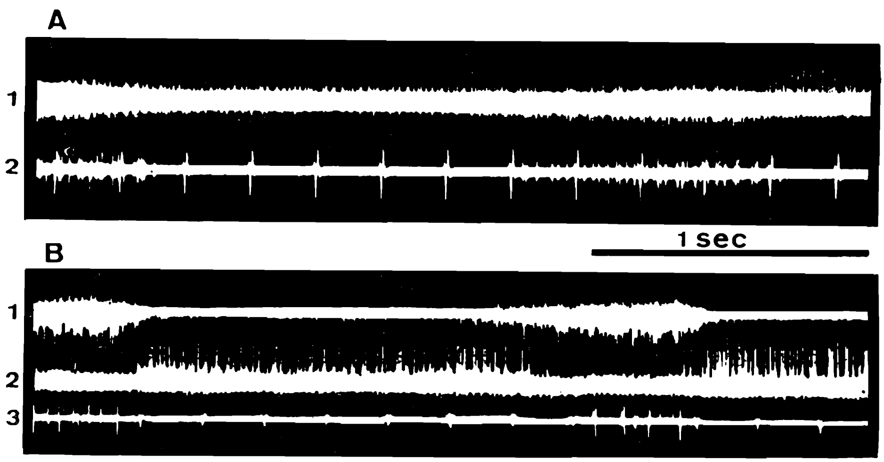

Fig. 1. Afferent activity recorded from an intact branch of the phrenic nerve during spontaneous respiration. *A:* Intact nerve. *1,* activity of the intact nerve; *2,* electrical activity of the diaphragm. *B*: After nerve section. *1,* central cut end; *2,* peripheral end; *3,* electromyogram of the diaphragm. The afferent discharge shows a typical silent period which persists in the peripheral end after nerve section; from time to time the afferent discharge presents an inhibition related to the cardiac cycle.

Dissection of the phrenic nerve branches

To facilitate the dissection and the insertion of the electrodes a large opening was used. The sterno-cleido-mastoid muscles, the sterno-hyoids and the most rostral portion of the pectoral muscles were cut at the level of the sternum and then retracted. The nerve were desheathed under a binocular dissecting microscope (Wild M 5). During the entire dissection the activity of the phrenic nerve was monitored via a loudspeaker which permitted recognition of any damage to the nerve during removal of the sheath. An oil bath was prepared from a small fold of skin in which the isolated nerve was submerged. To avoid spontaneous movements in the decerebrate preparation the cervical nerves and the branches of the branchial plexus were cut. The electrical activity recorded from the diaphragm and the nerve were amplified by differential amplifiers (Grass P 511) and fed to an oscilloscope (Tektronix RM 565).

A Grass kymographic camera was used to photograph the screen of the oscilloscope. By convention the amplifier and oscilloscope were connected in such a way that negative polarity applied to one of the amplifier inputs produced a upward deflection of the oscilloscope beam. Thus, when the distal hook of the bipolar electrode was connected to the input the afferent influx was recorded as a biphasic potential in which the principal deflection was upwards on the oscilloscope. The efferent influx caused an opposite deflection. Various methods to modify diaphragmatic tension were applied systematically after vagotomy; these were digital pressure to the diaphragm (direct stimulation) inflation or deflation of the lungs (indirect).

RESULTS

General aspects of activity recorded from the nerve

In each experiment at least one of the four branches of the right and left phrenic nerve yielded a variable type of electrical activity in addition to the characteristic motor discharge. These activities consist of an initial principal deflection in the opposite direction to the corresponding potentials of the motor discharge. They are of afferent origin because they persist in the peripheral end after cutting the phrenic nerve. Three modalities of afferent discharge can be distinguished by their occurrence at various phases of the respiratory cycle.

Afferent activity during expiration

This type of activity (Fig. 1) occurred in 72% of the recordings. The volley begins after the end of inspiration and has a "silent period" during contraction of the diaphragm. The frequency of discharge is highly variable in different cats. It generally increases toward the end of expiration. In an experiment when a single unit was recorded the discharge frequency varied from 25 to 50 Hz between the beginning and the end of expiration. These afferent discharges (expiratory type) are very stable and can be studied for several hours.

Afferent activity during inspiration

In 25% of the recordings, a potential which had a deflection opposite in direction to that of the motor discharge was visible during inspiration. When these potentials have sufficient amplitude they are easily distinguished. When they fail to emerge sufficiently from the motor discharge, only examination of the peripheral end of the branches after cutting allows them to be clearly seen. Generally the frequency decreases toward the end of inspiration. In one case in which a single unit was recorded the frequency varied from 150 to 50 Hz between the beginning and the end of inspiration. These inspiratory afferent activities are much less stable than those of the preceding group. They can disappear spontaneously and are therefore more difficult to study.

Activity independent of inspiratory activity

In a small number of recordings (3%) afferent activity could not be classified. Some are afferent volleys of the expiratory type which for unknown reasons (possibly changes in anaesthesia level) become progressively fragmented. Other discharges appear in rhythmic bursts that are unrelated to cardiac rhythm and which occur at twice the respiratory cycle frequency. These bursts are composed a few high frequency potentials and have a tendency to disappear quickly.

DISCUSSION

The activity recorded and described in this study leaves little doubt concerning the afferent nature of the "expiratory" and "inspiratory" volleys, since the activity persists in the peripheral end of the nerve after cutting it. The discharges that appear to be independent of the respiratory cycle probably have an afferent origin as well. Those recorded from the intact nerve show the same pattern as that described by Glebovskiï (1962) for the peripheral end of the cut phrenic nerve and some of these could be the pericardial fibres described by Rückebusch (1961).

Section of a single branch of the phrenic nerve does not modify the afferent discharge to any great degree. Our results from the intact nerve are quite similar to those of Glebovskiï (1962) and Cuenod (1961) from the cut phrenic nerve. This finding suggests that the partially denervated diaphragm retains a homogeneous contraction, despite a diminution of its electrical activity. This might be because the diaphragmatic motor units occupy a rather large area and they are intermingled with each other (Krnjević and Miledi 1958, Yasargil 1967, Duron and Condamin 1968).

The "expiratory" type of afferent discharge is that most frequently observed in this study. They most probably come from slowly adapting receptors because their discharges become continuous during a prolonged traction on the diaphragm. The fact that they present a "silent period" during diaphragmatic contraction favours their fusal origin. This silent period does not arise from differences in levels of anaesthesia due to a depressant effect on gamma motoneurons emphasized by Crowe and Matthews (1964*ab*). Thus their activity persists in the decerebrate non-anaesthetized cat. Crowe and Matthews (1964*ab*) have also shown that stimulation of static or dynamic fusimotor fibres eliminates the silent period of spindle discharges during passive relaxing. The activity of phrenic gamma motoneurons may therefore be insufficient to counterbalance the passive relaxing of the spindles during the muscular contraction. According to Merton (1953), muscular stretching excites primary terminals which in turn excite monosynaptically the alpha motoneurons, until the muscular contraction brings the muscle back to its original length. Thus during expiration most of the diaphragmatic spindles would be progressively stretched. The afferents activated would contribute to the depolarization of alpha motoneurons and would facilitate inspiration which would compensate the stretching of the muscle. During the silent period the diminution of the afferent volley would favour the interruption of the motor discharge.

The inspiratory type of afferent discharge

This also appears to be related to slowly adapting receptors, as has been shown by prolonged stretching of the diaphragm. Their origin is difficult to determine. They might be related to the tendon receptors which are known to exist (Dogiel 1902, Winckler and Delaloye 1957, Euler and Peretti 1966). They might equally be of spindle origin. Their activation during muscular contraction would then depend on gamma motor control. This would agree with the results of Eccles et al. (1962), Sears (1964) and Andersen and Sears (1970) who have shown that the rhythmic activation of alpha and gamma motoneurons is controlled by the respiratory center. The relative rarity of this type of discharge leads one to think that they do not play a very significant role in the regulation of breathing.

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