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<th>SPONTANEOUS EFFERENT IMPULSE AND REFLEX RESPONSE OF THE RECURRENT LARYNGEAL NERVE IN THE CAT AND RABBIT</th>
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In the recurrent laryngeal nerve, the action potential in respiratory rhythm was investigated by Green & Neil (1955) with the following results. The recurrent laryngeal nerve contains fibres which show an outburst of impulses during inspiration and also contains fibres which show an impulse traffic during expiration. The inspiratory and expiratory discharges of the nerve are respectively inhibited and accentuated by artificial inflation of the lungs. The afferent fibres of this reflex are vagal. According to the electromyographical study on the intrinsic laryngeal muscles, the recurrent laryngeal nerve innervates all intrinsic laryngeal muscles except the cricothyroid muscle and since all intrinsic laryngeal muscles represent an outburst of impulses in respiratory rhythm, they belong to the respiratory muscles in broad sense. Some investigators reported that the nerve innervating the intrinsic laryngeal muscle had an unimodal fibre-size distribution with a sharp about 10 to 12 μ and this fact suggested that the recurrent laryngeal nerve had no small motor fibre system.

These investigations suggest that records could be, in cat, taken from the central end of the recurrent laryngeal nerve as an index of respiratory activity without disturbing activities of proper respiratory muscles. From this standpoint, in order to explore the activity of the respiratory centre in diffusion respiration, efferent impulses of the recurrent laryngeal nerve were recorded by Joels & Samueloff (1956). In this paper, are reported further investigations of the efferent spontaneous discharge and reflex response of the recurrent laryngeal nerve.
METHODS

The experiments were performed on forty-four cats weighing from 1.0 to 4.8 kg and five rabbits weighing from 1.9 to 3.0 kg. The cats were anaesthetized with urethane (1 g/kg body weight) given intraperitoneally, or they were decerebrated at the precollicular level after induction with ether. In some experiments the cats were anaesthetized with Ravonal (thiopental sodium, 25 mg/kg body weight), given intraperitoneally. The rabbits were used as precollicular decerebrate or intact animals. The animals were fastened in a supine position and then a midline incision was made on the ventral surface of the neck. The trachea was cannulated using a Y-shaped cannula. The recurrent laryngeal nerve, usually the right one, was dissected free from the side of the trachea and cut peripherally at the point of entrance into the larynx; then the central end was dissected with a sharp needle on a small platform of black glass, to obtain small strands of dissected nerve and a slip containing one active single fibre. The prepared nerve was laid on Ag-AgCl electrodes, which were wound around with a single layer of cotton to keep a layer of TYRODE's solution of the surface of the wire; electrodes were connected to amplifier and thence to electromagnetic oscillograph or dual-beam cathode-ray oscilloscope.

Using electromagnetic oscillograph, an electroneurogram, electrocardiogram and pneumogram were recorded simultaneously on a moving bromide paper. The pneumogram was photographed on the change of tracheal pressure. In the cathode-ray oscillographical records, the electrodes were connected with a dual-beam cathode-ray oscilloscope unit and a loud-speaker; the second beam was used to record the chest movement. Simultaneously, the action potential and pneumogram were recorded photographically on X-ray film or moving bromide paper. For stimulation experiments, a square-wave generator was employed. The reflex responses were evoked by single shock of square-wave pulse (duration 0.05 msec, voltage 5 to 20 V). Silver wires used as stimulating electrodes which were applied to the central end of common peroneal or superior laryngeal nerve.

RESULTS

1. Spontaneous efferent discharge

When spontaneous efferent impulses were recorded from the trunk of the recurrent laryngeal nerve, the impulse discharges were observed continuously throughout the breathing cycle (Fig. 1.A). However, upon careful analysis of the records, it is noticed that the activity in the inspiratory phase was predominant in comparison with that in the expiratory phase. The predominance in the inspiratory phase became distinct on the records from the nerve filament (Fig. 1.B). When the functional single fibre preparations were obtained by repeated subdivision of the original nerve bundle, the discharge patterns indicated that the nerve contained three kinds of elements, viz., inspiratory, expiratory phasic and tonic (Fig. 2).

Investigation of the single active fibres showed that the majority of the phasic fibres indicated inspiratory activity. In addition, it is observed that, as inspiration proceeds, firing of individual phasic fibres accelerated in frequency, resulting in a progressive increment and the recruitment of new phasic elements. Duration, initiation and
FIG. 1. Spontaneous Discharges of the Recurrent Laryngeal Nerve

A B

In This and All Succeeding Figures, Inspiration is Upwards.

A. Nerve Trunk (urethanized cat)
B. Dissected Nerve Fibres (urethanized cat)

FIG. 2. Three Types of Active Single Fibres

A

B C

A. Inspiratory Element (urethanized cat)
B. Expiratory Element (urethanized cat)
C. Tonic Element (urethanized cat)
FIG. 3. Two Different Types of Inspiratory Elements
(urethanized cat)

FIG. 4. Two Different Types of Expiratory Elements
A. Urethanized cat  B. Decerebrate cat

FIG. 5. Two Different Types of Tonic Elements
(urethanized cats)
termination of burst discharge in the respiratory cycle varied in different phasic fibres (Figs. 3 & 4). Some of the tonic fibres sustained impulse discharge whose density was relatively homogeneous in the time course while other fibres increased slightly their impulse density in the inspiratory phase (Fig. 5). After respiratory movements were arrested by the neuromuscular blocking agent succinylcholine, the recurrent laryngeal nerve still exhibited rhythmic bursts (Fig. 6). In an early stage of the respiratory arrest, the discharge increased markedly while in a later stage, the interval of the rhythmic burst, which was accompanied with continuous discharge and grouping discharge, was prolonged. When spontaneous respiratory movements were arrested by succinylcholine, during the performance of artificial respiration, the rhythmic burst began in the early stage of the artificial inflation and terminated before the peak of the artificial inflation. The relations between the burst and artificial inflation were recognized when the artificial respirations were performed as the optimum frequency and volume (Fig. 7.A). Occasionally another burst followed in addition to the above-mentioned burst. In the increment of the artificial respiration frequency, the burst did not correspond to the artificial inflation (Fig. 7.B).

2. Reflex response to afferent stimulation

The experiments were performed with urethanized and decerebrate cats. When the homolateral peroneal nerves were stimulated by a single shock of square wave, the reflex responses of the recurrent laryngeal nerve were not clearly distinguished from the
spontaneous discharges in the urethaneized cats, but they were observed relatively clearly in the decerebrate cats. The latencies of the responses were about 16 to 27 msec. In addition, responses occurred occasionally whose latencies ranged from 35 to 42 msec (Fig. 8).

FIG. 8. Reflex Response to the Homolateral Common Peroneal Nerve Stimulus (decerebrate cat)

When the homolateral superior laryngeal nerves were stimulated by the single shock, the responses in the recurrent laryngeal nerve were observed more distinctly and the amplitudes were larger than when the common peroneal nerve were stimulated (Fig. 9A). Occasionally, oscillatory responses were obtained (Fig. 9B). The latencies were about 7 to 11 msec and the durations were about 8 to 14 msec.

FIG. 9. Reflex Response to the Homolateral Superior Laryngeal Nerve Stimulus (decerebrate cats)

DISCUSSION

The results obtained in the present investigations showed that the greater part of the recurrent laryngeal nerve fibres was inspiratory, as reported by Green & Neil and also that the nerve consists of three kinds of fibres, viz., inspiratory, expiratory phasic and tonic. Green & Neil reported that the inspiratory discharge of the recurrent laryngeal nerve occurred earlier by 0.1 to 0.2 sec than
the inspiratory discharge of the phrenic nerve. The results suggest that the inspiratory motor neuron of the recurrent laryngeal nerve is innervated by the inspiratory centre through fewer internuncial neurons than the innervation of the phrenic nerve. In the present experiments, no simultaneous records were obtained from the recurrent laryngeal and phrenic nerves. However, the present investigations showed that initiation of the burst discharge of the inspiratory fibre in the recurrent laryngeal nerve in the respiratory cycle varied individually. Therefore, it is concluded that the time relations to the discharge activity of the phrenic fibres are different in the individual inspiratory fibres of the recurrent laryngeal fibres and also that the inspiratory neurons in the recurrent laryngeal nerve are connected to the inspiratory centre respectively via the different numbers of the internuncial neurons. JOELS & SAMUELOFF reported that the recurrent laryngeal nerve still exhibited rhythmic burst discharge after respiratory movements were arrested by succinylcholine. The same results were obtained also in the present investigations. This fact means that the rhythmic burst discharges in the recurrent laryngeal nerve are not formed by the afferent influences of the HERING-BREUER reflex, but by the activity of the respiratory centre.

Histological findings\(^6\) showed that the motor cells of the laryngeal nerve exist in the nucleus ambiguus. On the other hand, ANDERSON & BERRY (1956) reported that antidromic potentials were recorded, when the vagus was stimulated, in the nucleus ambiguus which was often observed with a superimposed nuclear activity synchronous with the animal's respiratory cycle. Therefore, the motor cells of the recurrent laryngeal nerve exist undoubtedly in the nucleus ambiguus which is in the vicinity of the respiratory centre in comparison with the motor cells of the phrenic and intercostal nerves. According to the histological investigation by MURRAY (1957), there is the possibility that no small motor fibre system exists in the recurrent laryngeal nerve. Therefore, the efferent activity of the recurrent laryngeal nerve is not influenced probably from the proprioceptive reflex. From the above-mentioned facts, it may be concluded that the efferent activity of the recurrent laryngeal nerve represents most faithfully the activity state of the respiratory centre, observed in the peripheral nerve.

GREEN & NEIL reported that the inspiratory and expiratory discharges of the recurrent laryngeal nerve were inhibited or accentuated respectively by the artificial inflation of the lungs, as is known to occur in the phrenic and intercostal nerves. In the present investigations, when spontaneous respiratory movements were arrested by succinylcholine during the performing artificial respiration, rhythmic bursts synchronized with the artificial inflation were recorded. The synchronizations were observed only in the adequate frequency of the artificial
respiration. In the present experiment, the volume of artificial inflation was adjusted nearly at the same volume as the normal tidal air. Therefore, these results point out that the afferent impulses of the Hering-Breuer reflex are not the decisive factor in respect to the periodicity of the respiratory centre, but the modifying factor in the normal breathing.

In the present investigations, the reflex response in the recurrent laryngeal nerves exhibited short latent period ranging from 16 to 27 msec while the long latent period ranged from 35 to 42 msec when the common peroneal nerve was stimulated; the latency ranged from 7 to 11 msec when the superior laryngeal nerve was stimulated. Calma (1952) reported that a single afferent shock applied to the central end of the median nerve caused a motor discharge in the phrenic nerve whose latency was 19 to 22 msec, in the chloralosed cat, that the reflex response latency was 6 to 7 msec in homolateral and that it was 9 to 12 msec in contralateral in the spinal cat. Considering the conduction velocities, it may be concluded that the short latent periods in the peroneal stimulation in the present investigations, and the latent periods of the phrenic reflex described by Calma, were originated from the equivalent reflex arc in the medulla oblongata. On the other hand, it is estimated that the synaptic delay of the response in the medulla oblongata, following the stimulation of the superior laryngeal nerve is at the same order as the synaptic delay of the response in the phrenic nerve which was observed by Calma in the spinal cat.

**Summary**

1) The spontaneous efferent impulse and the reflex response have been investigated by recording the action potentials in the recurrent laryngeal nerve in the cat and rabbit.

2) The recurrent laryngeal nerve contains three kinds of elements in the discharge patterns, viz., inspiratory, expiratory phasic and tonic. Each group of elements has individually its various discharge types in respect to duration, initiation and termination of the burst discharge in the respiratory cycle.

3) When spontaneous respiratory movements were arrested by succinylcholine, the nerve still exhibited the rhythmic burst. When artificial respiration was carried on, the rhythmic burst began in the early stage of the artificial inflation and terminated before the peak of the artificial inflation.

4) In some experiments a single afferent stimulus of the common peroneal nerve caused a motor discharge in the homolateral recurrent laryngeal nerve. The latencies of the responses were about 16 to 27 msec. In addition, responses occurred occasionally whose latencies ranged from 35 to 42 msec.

5) When the stimulus was applied to the superior laryngeal nerve, the reflex
response was distinctly observed in all the experiments. The latencies were about 7 to 11 msec and the durations were about 8 to 14 msec.

**References**