EFFECTS OF ANTITUSSIVE DRUGS ON THE ACTIVITY OF THE RECURRENT LARYNGEAL NERVE IN CATS

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Abstract—Reflex responses in the recurrent laryngeal (RL) nerve to stimulation of the superior laryngeal (SL) nerve and the effects of antitussive drugs on these reflex responses were studied in order to elucidate the site of action of these drugs in encephale isolé cats. The RL nerve fibers were classified into four types by discharge patterns in respect to phases of artificial ventilation; type I: no spontaneous discharges, type II: discharges in phase with inflation of the lung, type III: discharges in phase with deflation of the lung, type IV: tonic discharges. Repetitive stimulation of the SL nerve elicited tonic burst discharges or short burst discharges in all types of RL nerve, while the same procedure inhibited ongoing discharges of the type III and IV fibers only. The inhibited fibers tended to have slow conduction velocities (20-80 m/sec). Administration of antitussive drugs such as codeine, dextro methorphan and oxymetebanol as well as pentobarbital decreased the frequency of the after spike discharges in the RL nerves evoked following repetitive shocks to the SL nerve, but had no effects on the inhibition of RL nerve discharges evoked by the stimulation of the same nerve. The neuropharmacological implication of these findings as related to the cough reflex are discussed.

The special role of the glottis in the cough is well known (1). The glottis closes for a short time and then suddenly opens very early in the process of expiration. The activity in the recurrent laryngeal (RL) nerve either opens or closes the larynx through contraction of the corresponding musculatures (2, 3). Histological and physiological studies have shown that the ambiguous nucleus is the motor nucleus of the RL nerve (4, 5). In a previous report, a preliminary intracellular study of the ambiguous neurons was performed and polysynaptic excitatory postsynaptic potentials (EPSP) from the superior laryngeal (SL) nerve were recorded (6). However, the small size of the ambiguous motoneurons impeded the stable recording of the synaptic potentials responsible for the contraction of the glottis during repetitive stimulation of the SL nerve, thus making a quantitative assessment of the change during the time course of drug action almost impossible. Therefore, the present study was undertaken to obtain more information on the motoneurons by recording activity of the motor axons in the RL nerve which induce contraction of the laryngeal muscles and to ascertain the effects of certain antitussive drugs and pentobarbital on the RL nerve in cats.
MATERIALS AND METHODS

The experiments were carried out on 20 adult cats weighing 2.5-3.5 kg. The animals were anesthetized with ether and fixed in a stereotaxic frame. The spinal cord was sectioned at the level of C1, and the animals were then maintained on artificial ventilation (rate 25-30/min., stroke volume 30-40 ml), by means of an intermittent positive pressure respirator. Tidal CO₂% was measured with a Beckman spinco LB 1 infra-red analyser, sampling from the tracheal cannula at 300-400 ml/min. The tidal volume was adjusted to maintain end-tidal CO₂% close to that during spontaneous breathing before paralysis (4-5%). Wound edges and pressure points were repeatedly infiltrated with 1% procaine.

Since the available anatomical evidence (7) showed that the majority of fibers coursing through the internal branches of the SL nerves were afferent fibers, the internal branches on both sides were exposed in the neck, sectioned near their entrance to the larynx and their central ends prepared for stimulation. The glossopharyngeal nerves on both sides were cut and freed from surrounding connective tissue for stimulation with bipolar silver wire electrodes. The vagus nerves on both sides were exposed and collar-type stimulating electrodes were placed around the nerves. In order to prevent the drying of the nerves and to keep them at or near body temperature, warm mineral oil was pooled within the cavity surrounding the neck muscles. The bath temperature was maintained with a radiant heater at 34-36°C. Body temperature was kept between 36°C and 38°C by a radiant heater from above and a heating pad placed underneath the animal. Arterial blood pressure was monitored through a cannula in the femoral artery. The respiratory movements were recorded with a thermister connected to one end of the tracheal cannula.

Activities of the nerve filaments were recorded by a pair of small platinum wire electrodes connected to capacity-coupled preamplifiers. The action potentials were displayed on a double-beam oscilloscope (VC-7, Nihon Kohden) and photographed on moving film with a kymograph camera (PC-1B, Nihon Kohden). Square wave stimulus pulses were obtained from an electronic stimulator (MSE-40, Nihon Kohden). The effects of drugs were studied on the discharge frequency of single units. The mean firing rate (per sec) of each single unit was calculated from the total number of spikes in a 4 sec period after repetitive stimulation of the SL nerve. Under good experimental conditions, the discharges of the same single RL nerve fibers evoked every 5 min could be recorded continuously for 1 hr or more without deterioration. However, in some cases, the changes in frequency range and mean frequency of the unit discharges evoked every 5 min by SL nerve stimulation from 5 to 30 min after the injection of the test drugs were adopted as a criterion of the drug effects.

Solutions of codeine phosphate and dextromethorphan hydrobromide were prepared by dissolving the drugs in distilled water. A new antitussive drug, oxymetebanol (8), was dissolved in 0.1 N hydrochloric acid. All of these drugs including pentobarbital sodium (Nembutal®) were injected into the femoral vein. The doses quoted in the text refer to
the salts except for oxymetebanol.

RESULTS

Spontaneous discharges of the RL nerve

The RL nerve, a branch of the vagus, carries motor fibers which discharge in synchrony with either inspiration or expiration (2, 3). However, in the present preparations which were maintained by artificial ventilation, we could only know whether the discharges were in synchrony with positive inflation or passive deflation of the lung. This preparation was suitable for the purpose of the present experiments in which the effects of the antitussive drugs on the SL-RL nerve reflex were observed without any anesthesia. Therefore, we classified the fibers into four types by discharge patterns in respect to phases of artificial ventilation: type I fibers showing no spontaneous discharge; type II fibers discharging in phase with inflation of the lung (Fig. 1A); Type III fibers discharging in phase with deflation of the lungs (Fig. 1B) and type IV fibers discharging tonically. Fibers of the latter type were not affected by either inflation or deflation of the lung (Fig. 1C).

Fig. 1. Spontaneous discharges of recurrent laryngeal (RL) nerves (upper beam) and tracheal airflow (lower beam, inspiration is indicated by a downwards deflection) in a "encephale isole" cat. A, fibers with discharges in synchrony with inflation of lungs (type II). B, fibers with discharge in synchrony with deflation of lungs (type III). C, fibers with tonic discharges (type IV). Time scale, 1 sec.

Of 156 RL nerve fibers recorded, 60 were classified into type I, 13 into type II, 13 into type III, 70 into type IV. Fig. 2 shows the frequency distribution of the conduction velocities of the RL nerve fibers and spontaneous discharges of the RL nerves. There was no clear relation between the sizes of the nerve fibers and the types of the fibers. The study did reveal that the type IV fibers tended to have slower conduction velocities (20–80 m/sec) than the other type fibers.

Discharges in the RL nerve evoked by single shocks to the vagus, glossopharyngeal and SL nerves

Electrical stimuli of near threshold intensities delivered to the cervical vagus evoked spike discharges in the ipsilateral RL nerve (Fig. 3A). These spike responses followed stimuli delivered to the cervical vagus nerve at 200/sec (Fig. 3B), and were obtained following the cut of the vagus nerve at the level of the central side from stimulating electrode. Therefore, these spikes were considered to be evoked directly. The latency of the RL nerve discharge varied over a wide range from 2.1 to 8.0 msec following a single shock to the RL nerve at bath temperature of 34–36 C. The average distance between the stimulation
Fig. 2. Conduction velocities and discharge types of the RL nerves in the "encéphale isolé" cat. Total number of RL nerves examined and total number of each type of fiber are indicated in white and black columns respectively. Type I: fibers with no spontaneous discharges; Type II: fibers with discharges in synchrony with inflation of lungs; Type III: fibers with discharges in synchrony with deflation of lungs; Type IV: fibers with tonic discharges.

Fig. 3. RL nerve discharges evoked by the stimulation of the ipsilateral vagus nerve (A, B and C), SL nerve (D, E, F, G and H), glossopharyngeal nerve (I) and contralateral SL nerve (J). Action potential of the vagus nerve evoked by a single shock (A) and repetitive shock at 200/sec. (B). Action potential and delayed spikes were induced by the stimulation of vagus at 3.4 NT (C). D-H indicated the response of RL nerve to graded SL nerve stimulation. Stimulus intensity, threshold in D, 1.5 NT in E, 8.5 NT in F, 9.6 NT in G, 26.8 NT in H: Note that the pause of spikes was induced in H. In I and J, stimulus intensities were supramaximal. Three or five traces were superimposed in each record. All traces were obtained in one and the same fiber which had no spontaneous discharges. Time scale, 1 msec for A and B, 4 msec for C to F and I, J, 20 msec for G and H. Voltage scale, 500 μV.
electrode and the active recording electrode was 22.5 cm. Hence, their conduction velocities ranged from 28 to 107 m/sec. If Hursh's proportion factor for the relation between the conduction velocity and the fiber diameter (9) holds for the RL nerve, its fiber diameter spectrum covers a wide range from 4.6 to 17.8 μ. These values are in accordance with the anatomical evidence that the RL nerve is a unimodal motor nerve with a preponderance of fibers having diameters of 10–12 μ (10).

The late spikes following the direct spike shown in Fig. 3C were evoked at a higher stimulus strength (3.1 NT, multiples of the threshold for the vagus nerve action potential). These late spikes failed to follow stimuli at 50–100 /sec and disappeared after the section of the cervical vagus nerve above the nodose ganglion. Hence, the late spikes were considered to be synaptically evoked via medulla oblongata. Electrical stimuli delivered to the ipsilateral SL nerve also evoked discharges multisynaptically in the RL nerve as shown in Fig. 3D-G. With the threshold stimulus only one spike was evoked (Fig. 3D). Upon increasing the stimulus strength to about 1.5 NT, three spikes were evoked (Fig. 3E). Maximal number of spikes was attained at stimulus intensities of 26.5 NT (Fig. 3F-H). The latency ranged from 7.0 to 15.0 msec with a peak at 10 msec. Of 154 RL nerve fibers examined with the stimulation of the SL nerve, only 3 type III fibers (see Fig. 1B) were not activated. Slow sweep displays of the RL nerve response after the SL nerve stimulation revealed delayed spike discharges. There was a pause between early and late spikes. The average latency and duration of this pause was 13.7 ± 0.44 msec (SE) and 32.3 ± 1.3 msec respectively for 20 fibers (Fig. 3H). Spontaneous discharges were also suppressed during this pause. Hence, this pause appeared to be inhibition of the RL nerve discharge by single stimuli to the SL nerve.

Some of the RL nerve fibers were also activated multi synaptically by the stimulation of the glossopharyngeal nerve (Fig. 3I). Of 166 fibers examined, 102 fibers responded to stimulation and the latency ranged widely from 7 msec to 16 msec. Some of the RL nerve fibers were also activated by the stimulation of the contralateral SL nerve (Fig. 3J). Of 156 fibers examined, 61 fibers responded to contralateral SL nerve stimulation. The latency for this route of activation ranged from 8 to 16 msec.

Discharge of the RL nerve evoked by repetitive stimulation of the SL nerve

The repetitive stimulation of the SL nerve at 5–50/sec for 2 sec elicited a tonic burst discharge or short burst discharge in the RL nerve during stimulation, which lasted well beyond the stimulation period. This effect was observed in all types of the RL nerve fibers.

**Fig. 4.** Effects of repetitive stimulation (20/sec) of the ipsilateral SL nerve (indicated by bars) on the RL nerves (upper beam). A, burst discharges of a RL nerve fiber were evoked. B, same procedure inhibited ongoing discharges in another RL nerve filament from the same preparation. Lower beam: tracheal airflow, inspiration is indicated by a downward deflection. Time scale, 1 sec.
Fig. 4A illustrates the results of one of these experiments (see also Fig. 6). In some of the type III and IV RL nerve fibers ongoing discharges were depressed by repetitive stimulation of the SL nerve (Fig. 4B). Fig. 5 shows the frequency distribution histograms of these fibers with respect to their conduction velocities. Of 156 RL fibers, 114 were facilitated, 15 were unaffected, and 27 were inhibited. These are indicated by gray, black, and white columns respectively in the figure. There was no clear relationship between the sizes of the nerve fibers and the type of the fibers as to whether they were facilitated or inhibited during repetitive stimulation of the SL nerve. However, the inhibited fibers tended to have slow conduction velocities (20–80 m/sec).

In five animal preparations, these reflex discharges evoked by the SL nerve stimulation remained unaffected after the transection of the brainstem through the lower border of the acoustic tubercle dorsally and the lower border of the trapezoid body ventrally. From this, it appears that the main part of the coordinating mechanism for producing the burst discharge and depressing the RL nerve must be localized in the medulla oblongata.

Effects of some centrally acting antitussive drugs on the burst discharges of the RL nerve

Intravenous injection of 1–5 mg/kg of codeine phosphate, dextromethorphan hydrobromide, oxymetetanol and pentobarbital sodium did not change the latency and number of evoked spikes recorded from the RL nerve by single shock to the SL nerve. However, these drugs did decrease the frequency of tonic spike discharges in the RL nerves evoked by repetitive shocks to the SL nerve. Typical results are shown in Fig. 6. Fig. 6A illustrates the reduction of the RL nerve fiber discharges evoked by a repetitive stimuli to the SL nerve, 5 min after intravenous injection of 4 mg/kg of codeine phosphate. In 5 RL nerve fibers, after administration of 4 mg/kg of codeine phosphate, the RL nerve discharges within 4 sec produced by repetitive stimulation of the SL nerve were decreased by 26.3 ± 4.3%. An injection of 5 mg/kg of dextromethorphan hydrobromide also produced a reduction in the discharges of RL nerve fibers as illustrated in Fig. 6B. The mean decrease of discharges was 24.2 ± 6.3% in 15 RL nerve fibers. Fig. 6C illustrates the depressive effect of oxymetetanol. Injection of 2 mg/kg of oxymetetanol produced a 36.8 ± 6.4% decrease in 5 RL nerve fibers. Subsequent injection of nalorphine hydrochloride reversed the effect of this drug on the RL nerve discharge evoked by repetitive stimulation of SL nerve. Injection
Fig. 6. Effect of antitussive drugs and pentobarbital on the evoked discharge of the RL nerve when the ipsilateral SL nerve was stimulated (indicated by arrows). A, control (upper beam) and 5 min after injection of codeine phosphate 4 mg/kg (lower beam) B, control (upper beam) and 5 min after injection of dextromethorphan hydrobromide 5 mg/kg. C, control (uppermost beam), 2 min after injection of oxymetebanol 2 mg/kg (middle beam) and the reversal of the decrease in discharges by the injection of nalorphine hydrochloride 0.5 mg/kg (lowest beam). D, control (upper beam) and 8 min after injection of pentobarbital sodium 10 mg/kg. Time scale, 1 sec.

Fig. 7. Effects of codeine phosphate (A), dextromethorphan hydrobromide (B), oxymetebanol (C) and pentobarbital sodium (D) on the evoked discharge of the RL nerve when the ipsilateral SL nerve is stimulated. Drugs injection times are indicated between the arrows. The mean firing rate (spikes/sec) of RL nerve fibers was calculated from the total number of spikes in a 4 sec period after repetitive stimulation of the SL nerve and the control value before administration of drugs was expressed as 100%. Note that the depressive effect of oxymetebanol was reversed by the injection of 0.5 mg/kg of nalorphine hydrochloride (NANM).

of 10 mg/kg of pentobarbital sodium abolished the burst discharge in the RL nerve. Even at this dose of pentobarbital, the single spike discharge was still evoked by repetitive stimulation to the SL nerve, as seen in the lower trace of Fig. 6D.
Fig. 7 illustrates the time course of the depression after various doses of these drugs on the RL nerve discharges evoked in a 4 sec period after repetitive stimulation of the SL nerve. Two mg/kg of codeine phosphate produced no decrease in the RL nerve discharge, but 4 mg/kg of this drug were effective for more than 40 min. Injection of 2.5 mg/kg of dextromethorphan hydrobromide produced a transient depressive effect, while 5 mg/kg of same drug was effective for more than 50 min. Oxymetebanol also produced a depressive effect and the effect was immediately reversed by the injection of 0.5 mg/kg of nalorphine hydrochloride (indicated as NANNI in Fig. 7). Injection of 5-10 mg/kg of pentobarbital sodium produced also a depressive effect for more than 30 min.

Table 1 shows the effect of these antitussive drugs on the discharge frequency of the burst of the RL nerve evoked in a 4 sec period after repetitive stimulation of the SL nerve. The frequency in the RL nerve burst discharges before drug administration was usually 20-60/sec. Intravenous injection of 4 mg/kg of codeine phosphate produced significant reduction of the burst discharges evoked by SL nerve stimulation from 5 to 30 min after the injection, to the mean frequency of 29.7±1.3/sec in 83 fibers. Intravenous injections of 5 mg/kg of dextromethorphan hydrobromide or 1 mg/kg of oxymetebanol also produced significant decreases in the mean frequency of the evoked burst discharges of the RL nerve to values of 24.5±2.2/sec in 41 fibers and 28.9±3.6/sec in 38 fibers respectively. These values were significantly lower (p<0.01) than the values obtained in non-treated animals. The RL nerve fibers inhibited by the repetitive stimulation of SL nerve were not affected by the antitussive drugs.

**Table 1. Effects of antitussive drugs on the discharge frequencies of the RL nerve**

|                | n  | Firing rate per sec |
|----------------|----|---------------------|
| Control        | 83 | 42.8±2.0            |
| Codeine        | 83 | 29.7±1.3            |
| Dextromethorphan | 41 | 24.5±2.2            |
| Oxymetebanol   | 38 | 28.9±3.6            |

Doses: codeine phosphate, 4 mg/kg; dextromethorphan hydrobromide, 5 mg/kg; oxymetebanol, 2 mg/kg. The mean frequency of the nerve fibers was calculated from the total number of spikes in a 4 sec period after repetitive stimulation of the SL nerve. The mean firing rates after drugs were calculated from records obtained 5 to 30 min after the intravenous injection of the drugs and were significantly lower (p<0.01) than the rates obtained in the non-treated animals.

**DISCUSSION**

Under the conditions of our experiments, the efferent activities of RL nerves depend on the rhythms of the respiratory center which are influenced by the pulmonary stretch fibers (11, 12). In these experiments, the activity patterns of the RL nerve fibers fell into four main groups with regard to the way they responded to the inflation or deflation of the lungs. A preponderance of type I and type IV fibers were recorded. The possibility was suspected at first that type I nerve fibers were injured ones. However, this was ruled out by
ANTITUSSIVE DRUGS ON RL-NERVE

the presence of evoked activity in this response to stimulation of the ipsilateral vagus nerve.

Single shock to the SL nerve and vagus nerve evoked action potentials reflexively in the RL nerve. The latency periods of the RL nerve discharges evoked by single shock to the ipsilateral SL nerve ranged from 7.0 to 15.0 msec with the peak at 10 msec. The mean latency was 10.7 msec. The average distance between the recording electrode on the RL nerve and the outlet of the vagus nerve from the medulla oblongata was 24.0 cm. Since the largest conduction velocity of the RL nerve was 107 m/sec, the conduction time between the outlet of the vagus nerve from the medulla and the recording site was calculated to be 2.2 msec. In a previous report concerned with a centrally induced cough (13), the nerve volleys were recorded with latency periods of 1.2 msec at the entrance of the SL nerve to the medulla oblongata. Subtracting this value from the latency of the RL nerve action potentials evoked by stimulation of the ipsilateral SL nerve, 3.6 (7.0 \( - (2.2 + 1.2) = 3.6\)) msec was obtained. This value should be central delay time and judging from this value, this central relay concerned with the SL-RL nerve reflex could be polysynaptic (cf. 5, 6).

According to Eyzaguirre and Taylor (14), repetitive stimulation of the central end of the SL nerve in the cat increased discharges of RL nerve fibers in phases of expiration which were inactive during eupneic breathing. They stated that the expiratory discharges, which influence closing of the larynx, probably play an important role in the cough reflex. On the other hand, the same procedure inhibited discharges of the inspiratory RL nerve fibers in their results. In our experiment, the RL nerve fibers of either type were activated by the stimulation of the SL nerve and the inhibited fibers were exclusively type III or type IV. It seems that type I and type II fibers play an important role in the cough reflex. This reflex discharge of the RL nerve was still recorded after the transection between the medulla and pons. According to Kasé et al. (15), the principal part of the fundamental coordinating mechanism for the cough induced by stimulation of the SL nerve is presumably localized in the medulla oblongata. If the reflex of the RL nerve from the SL nerve is included in the cough reflex as discussed by Eyzaguirre (14), the present results are in accordance with those presented by Kasé.

In view of this fact, it would be interesting to determine whether antitussive drugs affect the RL nerve discharge evoked by repetitive stimulation of the SL nerve. Engelhorn and Weller (16, 17) recorded the activity of expiratory neurons during coughs induced in anesthetized cats. They described that spikes of the expiratory neurons elicited by single shock to the SL nerve were abolished with 2 mg/kg of codeine. If the expiratory neurons described by Engelhorn and Weller were involved in the SL-RL nerve reflex in our experiment, it seems probable that such reflex was depressed by codeine. However, intravenous injection of antitussive drugs or pentobarbital in doses that suppress cough (18, 19) did not modify the evoked discharges of the RL nerve due to a single shock to the ipsilateral SL nerve. Therefore, in spite of the polysynaptic nature, the RL nerve reflex from the SL nerve was considered to tolerate depressants such as pentobarbital. However, the same doses of the antitussive drugs suppressed the increase in after discharges elicited by repetitive stimulation of SL nerve without change of the discharge during stimulation; while the RL
nerve fibers inhibited by the repetitive stimulation of SL nerve were not affected by same
doses of these antitussive drugs. Therefore, it is considered that these antitussive drugs
have no direct action on the RL nerve and motoneurons of the RL nerve, but act on other
unknown mechanisms increasing the discharge of the RL nerve. Although it is possible
that the activation of the RL nerve is due to the peripherally located receptors, it seems more
reasonable to assume that the drugs affect the medullary structure, on the basis of previous
studies which showed that drugs did not affect the peripherally located receptors (1). These
results are in good parallel with those of studies in which it was shown that the motoneur-
ons were activated by the stimulation of SL nerve and their evoked after discharges were de-
pressed by the injection of codeine and oxymetebanol without changes of spikes evoked
by single shock (20).

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