RE-EXAMINATION OF CENTRALLY-INDUCED COUGH IN CATS USING A MICRO-STIMULATION TECHNIQUE

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Received for publication March 25, 1972

Traditionally, the central structure for producing a cough response by electrical stimulation has been called the cough center (1). In 1948, Borison (2) stimulated the dorsolateral region of the medulla oblongata in the cat and induced a spasmodic respiratory response very similar to coughing, sneezing and retching. Chakravarty et al. (3) induced the same response by stimulating the same area and indicated the application to evaluation of antitussive drugs. Recently, Kase and his associates (4) explored a more caudal portion of the medulla and found a region capable of inducing cough-like response at the level of the obex, and the response induced appeared to be a little different in nature from the gasp-like responses observed by Borison. In these studies relatively large electrodes and high voltage stimuli (1.2-8.0 V) were used (5-7). Methodological limitations in these studies might have misled to assess a wider boundary as an area of the cough center. In the present article we have examined the area in the medulla by using a stimulating electrode of smaller size and a minute intensity of stimulus to induce cough-like response. The results indicate that the area, in which the cough-like response is produced by electrical stimulation, is confined distinctly to a certain anatomical structure of the medulla, i.e., the nucleus tractus solitarius.

METHODS

Twenty cats, weighing 2.5-3.5 kg, were used. These were anesthetized with ether under open drop method and fixed with the aid of a stereotaxic instrument. An occipital craniotomy was performed to expose the cerebellum, and portions of the cerebellum covering the medulla were removed by suction. Immediately after the surgical operation, ether inhalation was discontinued and the animal was kept under light anesthesia with pentobarbital sodium (15 mg/kg i.v.) alone. The trachea was cannulated at the level below the cricoid cartilage. The internal branch of the superior laryngeal nerve (SL nerve) was cut bilaterally at the entrance to the larynx and was separated carefully from surrounding tissues about 3 cm long up to the nodose ganglion. The distal end of each was ligated with a cotton thread and tied to the distal lead of a collar-type electrode implanted for stimulation and recording. A monopolar tungsten microelectrode (electrolytically polished, about 2/μ in diameter at the tip, insulated with polyurethane) was used for stimulation of the medulla. A silver-silverchloride plate was fixed at the neck muscle and was used as an indifferent electrode.
electrode was inserted into the medulla stereotaxically with a tilt of 20 degrees rostrally. Rectangular pulses were delivered from an electronic stimulator (Model MSE-40, Nihonkohden). The medulla was explored systematically: the electrode was moved step by step horizontally at 1.0 mm intervals and vertically at 0.2 mm intervals. The number of total penetrations in an animal was limited to be less than ten in order to avoid complications in identifying the sites of stimulation. Electrical stimulus of 1 msec pulses with 1-10 μA intensity, presented at a rate of 20 cps in a train of 5 sec duration. Train pulse stimulation with an intensity of about 10 μA was first applied to the locus to be tested and when a cough-like response was obtained, the intensity was lowered step by step to determine the threshold for the response. The intensity of current was measured as an IR drop through a 5000 ohm resistor placed in series with the microelectrode. At least 5 min was allowed to elapse between applications of the stimuli, because the response was noted to decrease when the stimuli were repeated with an interval of less than 3 min. Criterion for a positive response was immediate explosive expirations upon stimulation with pulses of intensity less than 10 μA. Negative responses were acceptable only if positive cough-like responses were produced from other medullary sites in the same animal. Respiration was recorded by airflow thermistors placed in a tracheal cannula and an inkwriting oscillograph. Arterial blood pressure was monitored through a cannula in the femoral artery. Electro-myogram of the rectus abdominis muscle was also recorded on the chart by a pair of needle electrodes inserted into the lateral part of the muscle. Threshold for the antidromic activation of the SL nerve by stimulating the medulla was also determined; a single pulse with an intensity of less than 10 μA and the duration of 1 msec was applied every 2 seconds through the microelectrode. The antidromically evoked action potentials of the SL nerve were led through a cathode follower to a dual beam cathode-ray oscilloscope (Model VC-7, Nihonkohden). Body temperature was kept between 36°C and 38°C by a radiant heater from above and by a heating pad placed underneath. In order to confirm location of the tip of the electrode, a DC current was passed through the stimulating electrode track. The brain stem was removed to make serial sections of 20 μ in thickness and stained with cresyl violet. The reactive points were identified with the aid of microprojection apparatus to make the composite map from the serial sections obtained from individual animals. As a reference antitussive agent, codeine phosphate was given intravenously.

RESULTS

1. Comparison of the cough-like response induced centrally with that induced peripherally

Cough-like response induced by a direct stimulation of the medulla is shown in Fig. 1 in comparison with that evoked by the SL nerve stimulation. Both responses consist of a series of spasmodic inspiration and explosive expiration of a large amplitude occurring at the rate of one or two per second, as seen in the tracing of respiration in the Fig. 1. When stimuli of moderate intensity were applied, changes in respiration began within 1–2 sec after the onset of the stimulation and were sustained as long as the stimulation was continued and often followed by an after-effect (see. Fig. 1B). In the responses observed in 12 cats,
there were no noticeable differences in patterns between the centrally and peripherally evoked cough-like responses, although the frequency of expiration in the centrally induced response was a little higher than that in peripherally induced one.

2. Medullary spots for inducing cough-like response with the minimal current stimulation

Areas which induce the cough-like response were explored by systematic application of stimulation in the medulla by a tungsten electrode. Seventy penetrations were attempted in 11 cats (Figs. 2 and 3). The positive sites were confined to an area extending from 3 mm rostral to 2 mm caudal to the obex and 3 mm lateral to the midline. The lowest currents for evoking the cough-like response obtained in each penetration averaged 5.9±2.4 (SD)
μA. The positive points (shown by open circles) identified histologically were projected on the dorsal surface of the IVth ventricle in Fig. 2. The positive points were distributed in the area from the level of nucleus olivaris inferior accessorius dorsalis (P=11) to that of the pyramidal decussation (P=16), as seen in Fig. 3 A-F, in which sites examined were projected on the different levels of the coronal section. As shown in Fig. 3 the positive points are concentrated in the nucleus tractus solitarius (abbreviated hereafter as STN). No positive points were found in the lateral area of the medulla which Borison had confirmed to be

![Composite maps of the medulla oblongata of the cat showing points at which cough-like response was produced by stimulation. Terminology according to Taber (17).](image)

A : Nucleus ambiguus ; CI : Nucleus cuneatus lateralis ; Cm : Nucleus cuneatus medialis ; C : Nucleus gracilis ; Lem : Medial lemniscus ; Lrm : Nucleus lateralis subnucleus magnocellularis ; Oid : Nucleus olivaris inferior accessorius dorsalis ; Oim : Nucleus olivaris accessorius medialis ; Oip : Nucleus olivaris inferior principalis ; Pyr : Tractus pyramidalis ; S : Nucleus tractus solitarius ; Ts : Tractus solitarius ; VII : Nucleus nervi facialis ; X : Nucleus nervi vagi dorsalis motorius ; XII : Nucleus nervi hypoglossi.
positive^2. There was a tendency to locate positive points more easily in the rostral portion of the nucleus than in the caudal region.

3. Whether or not central cough-like response is due to activation of the SL afferents?

The result described in the preceding section raises a question that the SL afferents to the STN could have been activated by medullary stimulation. If so, the medulla would have no special structures regarded as the cough center. This possibility was tested by comparing the threshold for antidromic activation of the SL nerve with that for the cough-like response from medullary stimulation at each spot in the same preparations. Top tracings of Fig. 4 A-D illustrate the responses of the SL nerve evoked by the stimulation of the medulla with 4 different intensities of stimuli such as 1, 2, 6 and 8 μA, respectively, and concomitant changes in respiration are shown in lower tracings. The threshold for the cough-like response due to the stimulation of the medulla was 2 μA in this case, this being approximately equal to the current necessary for antidromic activation of SL nerve. The monopolarly recorded nerve action potential showed a single smooth hump with an average latency of 1.2 msec. In A, the current applied (1 μA) was below threshold for both cough-like response and nerve action potential. In B, an immediate expiration and a small nerve action potential were observed with an intensity of 2 μA. In C, two phases of expiratory contraction were seen by 6 μA current, the amplitude of the nerve action potential was increased, but still one half of the maximum was attained by 8 μA current. In D, the repeated explosive expirations (cough-like response) at the rate of one per second were demonstrated by 8 μA current. Thus the current intensity for B was considered to be just

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**FIG. 4.** Relationship of antidromically evoked action potential in SL nerve and respiratory response after stimulation of the nucleus tractus solitarius of the cat. In A, B, C and D, stimulus intensity was 1, 2, 6 and 8 μA, respectively. SL nerve action potential was superimposed and was elicited by a single stimulus with 1 msec pulse to the brain stem. Respiratory responses in A to D were elicited by electrical stimulation: 1 msec., 20 cps, the same intensity as used for the antidromic activation of the SL nerve, for 5 sec.
above the threshold for both responses. The nerve action potential of the SL nerve could be elicited with repetitive stimulation of frequencies over 500 cps. Further the amplitude of the SL nerve responses remained unchanged even 2 min after a large dose of pentobarbital. Thus the nerve response was considered to be directly evoked. From the latency of antidromically evoked action potential and the conduction distance of 6.0 cm along the SL nerve, the conduction velocity of the SL nerve was estimated to be 23–47 m/sec, which coincided with reported values for the afferent fibers in SL nerve (8).

Threshold current for evoking cough-like response and SL nerve activation was attempted to measure in and around the STN in 10 preparations. Threshold for cough-like response was determined at 200 µA intervals with an electrode perpendicularly inserted. The threshold for cough and nerve action potentials obtained in one experiment is shown in Fig. 5. As the electrode was advanced from the floor of IVth ventricle through STN to the deeper structures, a sharp drop of thresholds was found for both cough-like response and SL nerve potential in the STN at 1.4 mm from the surface. The mean value of the lowest threshold currents or antidromic activation of the SL nerve obtained along each electrode track in 10 penetrations in 10 cats was 1.5 ± 0.5 (SD) µA. In each case, the area evoking cough-like response and SL nerve action potential at the minimal threshold intensity coincided and localized within the STN. Also seen was the tendency for positive sites to be located mainly in the ventral portion of the STN. The threshold for antidromic activation of SL nerve was clearly lower than that for evoking cough-like response, 5.9 µA, in the same area. This fact strongly suggests that the cough-like response from thalamus stimulation is due to direct activation of afferent from the SL nerve.

4. Effect of codeine on the centrally induced cough-like response

In order to clarify the characters of cough-like responses induced by stimulation of the
FIG. 6. Effect of codeine on the cough-like response evoked by stimulation of medulla. Cough-like response was induced by electrical stimulus consisting of 1 msec pulses, presented at a 20 cps in a train of 5 sec duration. Cough-like responses were alleviated at 5 and returned completely to the pretreatment state 40 min after i.v. administration of 2.0 mg/kg of codeine phosphate. Abbreviations are the same as in Fig. 1.

medulla, the effect of codeine was examined in an experiment outlined in Fig. 6. Centrally-induced cough-like responses were depressed for 30 to 60 min by intravenous doses of 2.0 mg/kg. Thus, it became clear that the depression occurred in the same mode of action as shown for the case of peripherally induced coughs (9).

DISCUSSION

In 1948, Borison demonstrated, by stimulating the medulla with a bipolar electrode, that the responsive sites were distributed over a rather wide area in the dorsolateral medulla including the STN and extended to more lateral regions of this nucleus, but were restricted to rostral part above the outflow of the 9th and 10th cranial nerves. Moreover, Kasé induced a cough response different from the spasmodic respiratory response of gasp-like nature of Borison’s observations by stimulating the lower part of the medulla. This response was depressed by codeine in such a dose as to depress the cough response induced through the SL nerve stimulation.

The present investigation has revealed that the sensitive area responsible for cough production is confined to an area in the STN region which extends longitudinally about 5 mm, from the level 3 mm rostral to 2 mm caudal to the obex level. It may be inferred that the regions reported by Kasé are included in the lower half of the responsive medullary site found in the present study. The responsive sites are considered to extend rostrally and caudally further along the STN. Stimulation of the area outside the STN in the medulla, however, was ineffective in producing cough responses even with an intensity of 10 μA, which was 5 times higher than the threshold. The STN was the only site evoking cough responses by weak stimulation. This site showed the lowest threshold also for the antidromic activation of the SL nerve. The cough responses elicited from these sites in our experiments
were also susceptible to the same small dose of antitussive agents as used by Kasé. Therefore, it appears that some of the Borison's results are due to excitation of the primary afferents as discussed affirmatively in his paper.

Gunn et al. (10) reported that a cough-like respiratory change was elicitable from the nucleus ambiguous in cats and dogs. They also evoked the diving reflex from the STN, i.e., respiratory arrest and cardiac slowing. In the present experiment, however, coughing was not evoked when the nucleus ambiguous was stimulated. Further, respiratory arrest was observed only when the STN was stimulated with more than 10 μA. This discrepancy may be ascribed to differences in the electrodes and stimulating strength employed.

In the present study, elicitation of cough by medullary stimulation failed without concomitant activation of afferent fibers. This strongly suggests that the cough reflex from the medulla oblongata is mainly due to direct activation of afferent fibers from the SL nerve. It is possible, however, that the neurons in this region and the incoming afferent fibers other than those from the SL nerve were activated concomitantly. Histological studies indicated that both thoracic vagus afferents and glossopharyngeal nerve terminated in the STN (11, 12). It was also reported that an evoked potential with short latency was recorded in the STN by stimulation of the thoracic vagus nerve (13). The slightly higher frequency of expiratory rhythm in cough response evoked centrally as compared with that evoked peripherally by SL nerve stimulation may be due to a possible concomitant activation of these structures. It may be stated however that there is no distinct neuronal cluster activated and solely responsible for the cough by such a weak stimulation as employed in the present study. The neurons responsible for coughs may be scattered diffusely in the medulla. The observation (to be published) that neurons in the STN simply followed SL nerve stimulation with a single or at most double spikes may also indicate that interneurons concerned with the formation of cough reflex, a rhythmic and tonic outflow to the peripheries, are not localized in the medulla. Recently Morest reported that the STN neurons have synaptic connections with the nucleus ambiguus and its adjacent regions (14). Engelhorn and Weller have shown that ambiguous neurons discharge tonically during the SL nerve stimulation in cats (15, 16). It may be conceivable that some of the neurons in and around the nucleus ambiguous play an important role in the cough reflex.

SUMMARY

The medulla oblongata was explored with microelectrodes in the cat to determine the area producing cough. Active loci capable of evoking the cough with the minimal intensity of current are localized in the nucleus tractus solitarius. The cough could not be elicited by medullary stimulation without concomitant activation of afferent fibers suggesting the possibility that the cough reflex from the medulla oblongata is mainly due to direct activation of afferents from the superior laryngeal nerve.

Acknowledgements: Gratitude is due to Dr. K. Kubota for advice and encouragement and also to Mr. T. Hara for excellent technical assistance.
REFERENCES