FUNCTIONAL DIFFERENTIATION OF HYPOGLOSSAL NEURONS IN CATS

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Intracellular potentials of hypoglossal motoneurons and the characteristics of their response to antidromic, as well as to orthodromic, stimuli, were described recently\textsuperscript{15,16}. Some questions remain, however, whether all hypoglossal neurons respond in the same manner to these stimuli, whether axon collaterals contribute to the inhibitory postsynaptic potentials of the motoneuron following stimulation of the hypoglossal nerve, and whether afferent fibers exist in the hypoglossal nerve.

In order to obtain further insight into these questions the present work was intended to examine: (a) the characteristics of hypoglossal neurons on the basis of the response to stimulation of the hypoglossal nerve and (b) the differences of response pattern or of the spontaneous intracellular potential changes of individual hypoglossal neuron. The present study may provide some proof for the coexistence of moto- and interneurons in the hypoglossal nucleus.

METHODS

Forty-eight adult cats weighing in excess of 2.0 kg were used for the experiments. In all cases the animals were decerebrated at midcollicular level by suction and were tracheotomized to insure adequate ventilation. The procedure was carried out under temporary ether anesthesia. By an approach through a dorsal midline cutaneous incision from the vertebral crests to the lambdoidal ridge, the hypoglossal nerve was exposed ipsilaterally for as long a length as possible, and severed. The nerve was then isolated from the surrounding tissues and immersed in vaseline to prevent drying and to insure electrical insulation during nerve stimulation. The stimulating electrode was a pair of platinum wires, 2 mm apart, on which the proximal end of the hypoglossal nerve was placed. Most of the muscular and/or tendinous connections between the skull and the cervical vertebrae were sectioned to minimize the movement of the brain stem.

The animal was rigidly fixed in a Horsley-Clarke apparatus and the vertebrae from Cl to C6 were also fixed tightly with special bars. Resections of the posterior-median part of the skull, partial decerebellation, cervical laminectomy and the posterior rhizo-
omy from C1 to C4 were carried out. Throughout the procedures, both carotids were untouched. The dura mater was opened widely and the edges pinned at several points bilaterally. After bleeding had ceased the exposed floor of the fourth ventricle and the surfaces of the cervical spinal cord were covered with 2% agar in Ringer's solution. The region of the left hypoglossal nucleus in the medulla oblongata was explored, using glass capillary microelectrodes, while electrical pulses of 0.2-msec duration were delivered at the rate of 2/sec to the central stump of the ipsilateral hypoglossal nerve. Microelectrodes of Pyrex pipettes filled with 3 M-KCl and the tip resistance between 20 and 80 megohms were used. Using a compensated cathode-follower, potentials were led off and displayed on two dual beam oscilloscopes, the one directly coupled and at low amplification for photography, the other condenser coupled at high amplification for visual monitoring. In addition to this, the routine auditory monitoring was also employed.

Intracellular recordings were made from various neuronal elements in the hypoglossal nucleus: motoneurons were identified by certain features of antidromic spikes produced when their axons were stimulated\(^1\). Spontaneous changes in intracellular potentials and the effects of stimulation with various parameters applied to the hypoglossal nerve were then studied. Artificial respiration was not employed unless signs of insufficient ventilation were definite. In a few animals paralysed by Flaxedil (3 mg/kg, i. v.), the effects of asphyxia on these potentials were examined. Continuous records were taken as well as stationary frames. These frame records were superimposed photographically when required afterwards.

At the end of each experiment the animal was sacrificed and the extramedullary distance of the hypoglossal nerve for antidromic conduction was measured. From this measure and the latency for the initiation of the antidromic spike potential, the conduction velocity and the diameter of individual motor axons could be calculated.

RESULTS

Electrical stimuli to the hypoglossal nerve set up a typical negative wave in the ipsilateral half of the medulla oblongata corresponding to the territory of the hypoglossal nucleus. The extent of this territory and the nature of the potential were in good agreement with previous descriptions\(^1\). As the electrode tip advanced through the medullary region which exhibited the evoked potential, a number of neural elements were penetrated. Thus, sharp spikes arose suddenly out of the negative wave of the evoked potential. These were unitary potentials from the cell bodies or from afferent or efferent fibers of the nucleus. In the amount thus detected motoneurons could be identified on the basis of criteria established for the spinal cord\(^1\). Without the peripheral electrical stimulation, most of the hypoglossal motoneurons were silent unless they deteriorated to yield a continuous train of injury discharges. A few cells, however, when encountered, showed "spontaneous" activity of nearly random occurrence or of the respiratory rhythm locked either with inspiration or expiration (Fig. 6). It was also clear that neurons of different types (see below) were found intermingled and revealed no tendency of organized localization within the hypoglossal nucleus. Of 158 neurons successfully penetrated,
149 were excited antidromically and the remaining 9 synaptically to the stimulation of the hypoglossal nerve.

1. Types of response to stimulation of the hypoglossal nerve
   a) Motoneuron responses. Antidromically driven action potentials of hypoglossal motoneurons were similar in configuration to those of motoneurons in the anterior horn of the spinal cord (Fig. 1A and cfr. 3). The duration of full-sized spikes of 149 motoneurons ranged from 0.93 to 2.6 msec with a mean

![Fig. 1. Antidromically induced spike potentials of a hypoglossal motoneuron (A) and diameter spectrum of motor nerve fibers of the hypoglossal nerve (B). A: full-sized spike revealing an inflection on its rising phase (a), IS spike (b) and M spike (c) are illustrated. Horizontal line with time marks indicates reference potential level for (a) exclusively and msec. Upward deflection indicates positivity in this and all other records. B: diameters of motor nerve fibers were calculated from responses of 149 hypoglossal motoneurons to antidromically applied shock stimuli (see text).](image-url)

- of 1.7 msec. The rising phase was 0.39 msec and the declining phase 1.33 msec. In the course of the spike potentials an inflection on the rising phase was evident. The sizes of resting potentials and action potentials ranged respectively from 41 to 83 mv and 33 to 79 mv in neurons considered to be in good condition. In many such cases, overshooting was noted, up to 23 mv in one motoneuron. During or after antidromic stimulation, with repetitive pulses, block of SD or IS spike was produced, so that the IS or M spike became prominent (Fig. 1Ab and c). The block was often elicited by stimulation of even low frequency (2/sec). No hypoglossal neuron was found in those antidromic and orthodromic activations which could both be produced by a
single shock to the hypoglossal nerve. The latency of antidromic invasion for 149 motoneurons was 0.27 to 2.34 msec with a mean of 0.98 msec. From this and the conduction distance of 2.3 to 3.6 cm along the hypoglossal nerve the conduction velocity (meter per sec) and hence, the diameter of motor fiber (in μ) could be estimated through dividing the value of velocity by six\(^{13}\). The distribution spectrum of the motor fibers in the hypoglossal nerve thus depicted is illustrated in Fig. 1B. It is clear that the hypoglossal motor fibers have a gross unimodal distribution, the peak lying between 3 and 5 μ. The prominent cluster of values in the range of 12 to 20 μ that can be seen in the diameter spectra of spinal ventral root\(^{8,21}\), is absent in the spectrum of hypoglossal motor fibers. It can be deduced, therefore, that the conduction velocity of hypoglossal motor axons is generally much slower than in axons of spinal motoneurons. The contour of the histogram resembles that obtained by direct measurement of the axon size of the hypoglossal nerve in stained cross sections\(^{2,22}\). There is, however, a minor difference between the results of the electrophysiological and histological experiments. The peak in the distribution histogram of the former is shifted in the direction of increasing size by 1 to 1.5 μ as compared to the latter. The difference might be attributed to the length of intramedullary portions of the axons which were not included in the length measurement in the present experiments.

In some cases, the motoneurons were excited antidromically with continuous trains of stimuli of various frequencies as well as with pairs of shocks. The block of SD and IS potentials ensued in turn as the frequency of the stimuli was increased or the interval of the paired shocks was decreased. The results of these experiments are exemplified in Fig. 2. If one takes the responses for the beginning 100 msec of the trains of neuron spikes, it can be seen that the hypoglossal motoneurons faithfully followed antidromic stimuli up to 90 to 100/sec before the block of SD spikes occurred, but if the later stages of each train of discharge, e.g., 5 seconds after the onset of the stimulation, were taken the critical frequency for the block was lower than 30/sec. The unit revealed blocking of every third stimulus at 50/sec and firing of every third at 100/sec and at 200/sec only of every ninth. Thus, the frequency of firing tended to maintain itself within the range of 20 to 25 impulses per second despite the increases in stimulus frequency. This may be partially accounted for as adaptation of the neuron. The block of IS spikes occurred at a higher frequency (300 to 350/sec) than for SD spikes. When the stimulus frequency was raised further up to 2000/sec, the IS spikes also tended to attain ultimately a frequency between 20 and 25/sec which was roughly equivalent to the discharge of SD spikes (Fig. 2A bottom trace). Hyperpolarization for over 100 msec occurred after each response. In several motoneurons, the critical frequency for blocking was 15 to 20/sec for SD spikes and 30 to 50/sec for IS spikes (Fig. 3).
Fig. 2. Effects of antidromic stimulation at various frequencies (A) and with paired stimuli of various intervals (B) upon a hypoglossal motoneuron. A: numbers at left of each record indicate frequencies of stimuli per second. Time bar: 1 sec. B: inflection, just visible on the rising phase of the first spike, was more pronounced in the second spike evoked by test stimulus applied 4.8 msec after the conditioning one (e). Blocking of SD spike occurred at 3.0 msec (d). The IS spike decreased in size until it disappeared at 1.3 msec (a). Time scale: msec.

Fig. 3. Effects of the antidromic stimulation with repetitive pulses upon two hypoglossal motoneurons (A and B). Numbers at left of each record indicate frequencies of stimuli per second. Time bar: 1 sec. Note the SD and IS blocking by the repetitive stimuli at 30/sec (A) and at 50/sec (B) respectively (cfr. Fig. 2A). Some spontaneously generated spikes are seen at the beginning of the bottom record.

The critical interval of the paired shocks that initiated disintegration between SD and IS spikes ranged from 3.0 to 6.0 msec. When the interval was reduced to less than this value, the second shock was followed only by an IS spike which decayed in amplitude and finally disappeared as the interval was
b) Interneuron responses. Of 158 nerve cells successfully penetrated and which responded to stimuli of the hypoglossal nerve, nine nerve cells did not reveal an antidromically induced spike potential but yielded responses thought to be synaptic in nature. The size of the spike potentials of these neurons was generally smaller than those of the motoneurons described above. The evident feature common to all these neurons was the outbreak of a burst of repetitive spikes in response to a single stimulus. There were, however, a few differences in the details of the responses between neurons. The first group of neurons (5 of 9 cells) responded after a latency of between 3 and 6 msec and revealed a lesser number of spikes (not more than 5/burst), a shorter burst duration and a prominent excitatory postsynaptic potential (Fig. 4A).

![Fig. 4. Two types of hypoglossal neurons fired repetitively by single hypoglossal nerve stimuli. Latencies of the responses were 3.0 msec (A) and 0.8 msec (B) respectively. In A, polysynaptically induced EPSP is prominent. Time marks: msec. Potential scale is applicable to both A and B.](image)

All of these features described, however, were not consistently present in every cell. The neurons of the second group (2 of 9 units) showed contrasting features as compared with those of the former; namely, shorter latency (less than 1.0 msec), greater number of spikes per burst, longer burst duration and lower amplitude of the excitatory postsynaptic potential (Fig. 4B).
Sizes of the spike potentials did not exceed 8 mv. The neurons were too fragile to continue recording of their activity and it disappeared in a few minutes, at which time no sign of injury could be detected. From these findings, although some other response characteristics such as their pharmacological behaviors are unknown, it may be assumed that they are similar to RENSHEW cells in the ventral horn nucleus of the spinal cord\textsuperscript{9,18}).

As shown in Fig. 5, a neuron categorized in the first group (although the potential seemed to be recorded extracellularly) was tested by paired stimuli to the hypoglossal nerve. The response to the test stimulus was augmented when compared to that elicited by the conditioning stimulus. This facilitatory influence was enhanced by further shortening of the pulse interval. These findings may give proof of the synaptic activation of the neuron and consequently of the presence of afferent fibers in the hypoglossal nerve trunk.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig5}
\caption{Responses of a hypoglossal neuron to a series of paired stimuli with various intervals applied to the hypoglossal nerve. In A, the spike number after the test stimuli is increased with gradual shortening of the pulse interval. The negative deflection following each stimulus artifact indicates the field potential corresponding to the antidromic invasion to the hypoglossal nucleus. Time marks: msec. B: plots for latency and number of spikes per response (ordinate) at various intervals between stimuli (abscissa). Note increased facilitation at shorter intervals.}
\end{figure}

C) Other types of neuron responses. Apart from those described above, two neurons responded in a peculiar fashion to hypoglossal nerve stimuli. The one discharged sporadically only during the period of repetitive electrical stimulation (Fig. 6A), the other discharged a burst of spikes to each stimulus (Fig. 6B). In both cases the spikes, when produced, constantly rode on the
Fig. 6. Responses of two neurons to the stimulation of hypoglossal nerve. In A, the stimulus frequency is 100/sec and a sporadic discharge of full-sized spikes, none of which exhibits any sign of antidromic activation, is clear. In B, single shock of equal strength produced burst responses of various degree. Top of the spikes in each burst was photographically excluded. Time marks: 0.1 sec (A), msec (B).

excitatory synaptic potentials. Moreover, the latter neuron exhibited instability of response latency, namely it varied within a wide range as from 9 to 30 msec to the equal strength of stimuli. The responses might not be of the hypoglossal neuron but were likely to be of an intercalated neuron receiving afferent inputs in the reticular formation immediately adjacent to the nucleus, the significance and character of the neurons were obscure. No neuron yielding pure inhibitory response by central stimulation of the hypoglossal nerve was identified.

2. **Spontaneous changes of intracellular potential in hypoglossal motoneurons**

a. Potential changes in hypoglossal motoneurons with respiratory rhythm. During progressive insertion of a microelectrode through the hypoglossal nucleus it was found, on some occasions, that the penetrated neurons produced bursts of action potentials, constantly coinciding with some phase of respiration. In two of these neurons successful recordings were made of the potential change during the respiratory cycle. Records from such a neuron are illustrated in Fig. 7. The neuron was a motoneuron since it was found in the middle of the nucleus as judged by the evoked potential and antidromic spike potential to stimulation of the hypoglossal nerve. The membrane potential, when measured in the interval between two successive bursts of action potentials, was 41 mv and the amplitude of action potentials ranged from 43 to 52 mv with an average overshoot of 6 mv.

From a point in the middle of the respiratory pause or just before a particular burst, the membrane potential first began to shift in the direction of depolarization and the first action potential occurred thereafter. Each action potential was followed by after-hyperpolarization or repolarization for a
few tens of millisecond and the membrane potential progressively shifted again in the direction of depolarization until the initiation of another action potential. These changes occurred repeatedly throughout the period in which action potentials were present. It can be seen in Fig. 7, however, that the firing level became less negative as the sequence of the burst of action potentials progressed. The maximal repolarization level of each action potential also revealed progressive depolarization during the burst activity. The membrane potential, after the last action potential of the burst, either continued to shift for a short period or declined immediately and returned within 200 to 300 msec to the level of maximal negativity. From this level the membrane potential again slowly shifted in the direction of depolarization until the first action potential of the next volley occurred. The maximal range in which the membrane potential changed during a respiratory cycle was from 8 to 10 mv.

b. Non-respiratory slow fluctuation of intracellular potential in hypoglossal motoneurons. Some hypoglossal motoneurons revealed slow potential changes whose rhythm was characteristic for individual neurons and which locked neither with the respiration nor the cardiac beat of the animal. The first type was of 1.8 c/sec with discharges of small spikes (presumable EPSP's) at
the maximum of each fluctuation (Fig. 8A). The frequency of the discharge was highest at the maximum of depolarization, 14 mv less than the original potential level in this case. At the nadir of fluctuation, no or very few spikes were produced. The height of the small spikes did not exceed that of the inflection on the rising phase of the antidromically induced full-sized action potential.

The second type of the slow potential change revealed 0.5 c/sec in frequency and 11 mv in maximal depolarization (Fig. 8B). The spike did not occur at the peaks of the fluctuations as they did in the first type described, however, a small fluctuation of 3.0 c/sec concurred throughout the course.

In the third type, as seen in Fig. 8C, fluctuations of 12 mv in the direction of hyperpolarization (that can be judged from the first deflection in the trace) is clear. This was produced when the animal was asphyxiated by interrupting the artificial respiration during motoparalysis.

DISCUSSION

Two types of nerve cells were found in the hypoglossal nucleus by stimulating the central stump of the hypoglossal nerve; motoneurons, identified by antidromic spike potentials; interneurons, including RENSHAW type cells, exhibiting excitatory postsynaptic potentials upon which were superposed a repetitive spike discharge. In the spinal ventral horn nuclei a motoneuron interacts with other types of neuron through axon collateral pathways. In the hypoglossal nucleus, however, the presence of such axon collaterals has never been observed either histologically or electrophysiologically. There is, however, a report by GREEN and NEGISHI, in which they suggested their presence, though lacking the positive evidence as shown in Fig. 4B. The failure and difficulty in finding the cell in the previous studies can be ascribed to the relative rareness of this type of cell in the hypoglossal nucleus.

In the spinal cord, tested by antidromic stimuli, phasic motoneurons have a higher firing rate than tonic ones, because they have less after-hyperpolarization. Analogous functional differentiation may exist also among motoneurons revealing SD-IS blocking at a rate from 90 to 100/sec (Fig. 2A), whereas another at 15 to 20/sec (Fig. 3). Two groups of hypoglossal motoneurons were also distinguished histologically, i.e., a more dorsal one in which the cells appeared to be fusiform with one or two dendrites in a given profile, and a more ventral group, in which larger cells of about 30 μ diameter were present. These physiological and anatomical findings may indicate the same fact but from different directions. Lack of hypoglossal motoneurons that exhibit short after-hyperpolarization of 40 msec in duration as observed with oculomotor neurons may suggest the difference in characteristics of contraction between muscles responsible for the movement of the eyeball and
that of the tongue.

It has hitherto been proposed by many investigators, and reconfirmed in the present study, that there are some afferent fibers\(^1\) in the hypoglossal nerve trunk which can synaptically excite the hypoglossal neurons. Both intracranial and intramedullary pathways for these afferents, however, could be diverse as postulated by PORTER\(^1\). In the present experiments, the possibility that the spread of stimulating current from the hypoglossal nerve electrode to excite low threshold afferent fibers in the lingual nerves as well as to contract the tongue receptors directly\(^1\) could be excluded by careful isolation and insulation of the hypoglossal nerve trunk (see Methods).

The conduction velocities of the hypoglossal nerve fibers could not be assessed precisely, since the length of their intramedullary course is uncertain. Rough estimation of the conduction velocities of the hypoglossal motor nerve fibers, however, gave values of from 10 to 90 m/sec (mean: 32 m/sec) and the histogram of diameter distribution of these fibers displayed an unimodal configuration as shown in FIG. 1B. These figures differ slightly from previous results in which the distribution shifted rightwards, towards increasing size, by 1 to 1.5 \(\mu\) to \(\mu\). The difference can possibly be ascribed to the intramedullary length of nerve fibers as described above.

The changes of intracellular potentials in phase with respiration in the hypoglossal motoneurons are in good correspondence with those in the medullary respiratory neurons\(^1\), the phrenic\(^1\) or thoracic\(^2\) respiratory motoneurons. Since the previous authors\(^1\) described that the potential recording from inside the medullary respiratory neurons was extremely difficult, and they did not confirm the precise intramedullary site of the neuron, their results might presumably involve those of the hypoglossal motoneurons as shown in FIG. 7 of the present report.

The significance and role of the rhythmical fluctuations of various rates occurred in the motoneuron membrane potential (FIG. 8) were yet unknown, although speculations may be possible in connection with the source of the "spontaneous discharge" of the neuron. It is not clear at present, however, whether the fluctuations are functionally homologous with the intrinsic rhythm of the human brain\(^2\) and with the pacemaker potential in ganglion cells of crustacea\(^4\).

**SUMMARY**

In decerebrate and unanesthetized cats, changes in the intracellular potential of neurons in the hypoglossal nucleus following stimulation of the hypoglossal nerve were recorded.

1. Of 158 cells examined, motoneurons (94.3%), interneurons (5.7%) were distinguished by hypoglossal nerve stimulation.
2. Two groups of motoneurons responded differently in critical frequency for SD and/or IS blocking to the antidromic stimulation.
3. Sizes of the motor axons were estimated from the conduction distance and latency of antidromic invasion to each motoneuron. The diameter distribution revealed gross unimodal configuration with the peak between 3 and 5μm.
4. Some of the characters of the potential change in the “respiratory” hypoglossal motoneurons and slow fluctuations of various rhythm in others were reported.

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