Neuronal RNA in Nucleus Ambiguus and Nucleus Hypoglossus of Patients with Amyotrophic Lateral Sclerosis

HENRIK A. HARTMANN, M.D., STEPHANIE McMAHON, B.S., DEXTER Y. SUN, M.D., JAMES H. ABBS, PH.D., and ETSURO UEUMURA, D.V.M., PH.D.

Abstract. To establish objectively the involvement of neurons in the medulla oblongata in patients with amyotrophic lateral sclerosis (ALS), ribonucleic acid (RNA) content was determined in neurons of the hypoglossal nucleus and the nucleus ambiguus. Neurons from these two nuclei showed a significant loss of RNA content in patients with ALS; only 57% and 38% of the normal RNA content was found in hypoglossal and ambiguous neurons, respectively. This marked loss of neuronal RNA suggests changes in functional states of neurons, which may contribute to fasciculations in the tongue and difficulties in swallowing often associated with ALS.

Key Words: Amyotrophic lateral sclerosis; Medulla oblongata; Motor neurons; Nucleus ambiguus; Nucleus hypoglossus; Ribonucleic acid.

INTRODUCTION

Muscle weakness of the tongue and pharynx are symptoms seen in patients with amyotrophic lateral sclerosis (ALS). One review of the histories of 668 patients revealed that only 19% had the bulbar type (1). In another review of 441 patients with ALS, 28% had primary head and neck symptoms of which the majority of patients had tongue fasciculations and slurred speech (2).

Neuropathological studies of specific nuclei showed a much higher degree of involvement than clinical symptoms had indicated. One earlier study revealed that the nucleus ambiguus was affected in 43 of 53 patients, or 81% (3), while another investigator found mild involvement in 68% of 26 patients (4). The nucleus ambiguus supplies cranial nerves IX, X, and XI with motor fibers which innervate the laryngeal muscles.

The above studies revealed changes in the hypoglossal nucleus in 50 of 53 patients, an incidence of 94% (3), while 26 patients showed severe involvement in 14, moderate in 2, and mild in 2, altogether a 90% incidence (4). The criteria for tabulating involvement were explained in terms such as “destruction of nuclear components” and “complete cellular destruction.”

To evaluate the functional state of the hypoglossal and ambiguous nuclei in patients with ALS, RNA content in single cells from those two nuclei was compared with RNA content in cells from approximately age-matched normal patients.

MATERIALS AND METHODS

Brain stems were obtained at autopsy from four patients who had no neurological diseases (ages ranging from 48 to 80, with a mean of 65) and four patients with ALS (ages ranging
TABLE 1
Mean Neuronal RNA Content

<table>
<thead>
<tr>
<th></th>
<th>Mean age</th>
<th>N</th>
<th>RNA (pg)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ambiguous neurons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 3)</td>
<td>61</td>
<td>72</td>
<td>297</td>
<td>15</td>
</tr>
<tr>
<td>ALS (n = 3)</td>
<td>60</td>
<td>60</td>
<td>112†</td>
<td>19</td>
</tr>
<tr>
<td><strong>Hypoglossal neurons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td>69</td>
<td>138</td>
<td>156</td>
<td>11</td>
</tr>
<tr>
<td>ALS (n = 4)</td>
<td>61</td>
<td>102</td>
<td>89*</td>
<td>22</td>
</tr>
</tbody>
</table>

N denotes number of cells analyzed.
RNA was determined for groups of six neurons and divided by six to give mean RNA per neuron.
* and † indicate p = 0.037 (df = 6) and p = 0.0015 (df = 4) respectively, by t-test.

from 42 to 74, with a mean of 63). The post-mortem interval varied from two to 12 hours (h). Fixation was done in 10% buffered formalin, for two to three weeks. The medulla oblongata was transversely sectioned at the level of the olives into four, 4 mm thick pieces. After further fixation and washing overnight in water, the sections were dehydrated in 90% and 100% ethanol for 90 minutes (min), cleared in benzene for 90 min and infiltrated in paraffin for eight to 12 h.

Ribonucleic acid (RNA) was determined according to the single cell method of Edstrom (3). Thick sections (80 µm) were cut from the paraffin blocks and mounted on half cover slips. After deparaffinization in chloroform, the sections were treated with 100% ethanol and hydrated in 0.01 M acetic acid. The tissue was blotted to remove excess water, and the cover slip inverted and placed in a deFonbrune dissecting chamber, where it was covered with liquid paraffin to prevent evaporation. Alternate 10 µm sections were mounted and stained with methylene blue to identify the nucleus ambiguus and the nucleus hypoglossus. From the 80 µm sections, single nerve cells were dissected free from the surrounding tissue using two micro needles which were controlled by a deFonbrune micromanipulator and viewed under a phase microscope at ×80 magnification.

A total of 138 and 102 cell bodies of hypoglossal neurons were dissected out from four control and four ALS patients, respectively. From the ambiguous nuclei of three control and three ALS patients, a total of 72 and 60 cell bodies were dissected out, respectively (Table 1).

Nerve cells were placed in groups of six and extracted in a drop of ribonuclease in a volatile buffer solution at 37°C for one h. The procedure was repeated three times. Each time the extract was evaporated onto a quartz coverslip. The extracts, when dissolved in a glycerol-containing buffer, formed lens-shaped drops, which were photographed in ultraviolet light at 257 nm together with a rotating step wedge for reference. The film was scanned in a densitometer and microgram (pg) quantities of RNA determined from integrating and calculating the areas under the peaks according to Edstrom (5). The mean neuronal RNA content was calculated by dividing the value for each drop with 6.

The data were assessed by t-test. In all cases, each patient was used as the unit of variance.

RESULTS

The nucleus ambiguus is normally composed of large, somewhat elongated multipolar neurons (Fig. 1) which provide motor innervation to the larynx and the pharynx (6). In patients with ALS the cell bodies of those neurons were very atrophic (Fig. 2) and apparently reduced in number (3, 4). By careful microdissection, we were able to isolate sufficient numbers of cell bodies and extract them with ribonuclease.

The RNA content of ambiguous neurons from patients without neurological diseases...
was 297 pg per cell body, whereas that from patients with ALS was 112 pg per cell body (Table 1). The difference in neuronal RNA content between the control patients and patients with ALS was highly significant ($t = 7.75$, df = 4, $p = 0.005$).

The hypoglossal nuclei in control patients were characterized by large, multipolar, motor-type neurons which contained conspicuous, deeply stained, geometrically shaped Nissl granules (6) (Fig. 3). In patients with ALS, the hypoglossal neurons were markedly atrophic and apparently reduced in numbers (Fig. 4). This was true in all patients with ALS.
The RNA content of hypoglossal neurons from patients without neurological diseases was 156 pg per cell body, whereas that from patients with ALS was 89 pg per cell body (Table 1). The difference in neuronal RNA content between the two groups of patients was significant ($t = 2.68$, $df = 6$, $p = 0.037$).

An attempt was made to correlate the above results with the clinical symptoms. When the histories of the six ALS patients used in this study were reviewed, the following symptoms were identified: weakness of extremities was present in 6/6 patients; speech difficulties were present in 5/6 patients; and swallowing difficulties...
were present in 2/6. In other words, bulbar symptomatology was present in only 2/6 patients, while neuronal RNA in the nucleus ambiguus and hypoglossus was reduced in 5/6 patients.

**DISCUSSION**

In these studies we tried to age match the patients with ALS to normal patients. It has been shown that RNA decreases with age in hypoglossal neurons (7) but that this is not the case in other neurons (8–10). We are not aware of any study showing what might happen in the neurons of the nucleus ambiguus with age. Our findings that the neurons of two of the most prominent motor nuclei in the medulla oblongata show decreases in RNA in patients with ALS, are in keeping with the older pathological observations (3, 4) that the motor nuclei are involved, regardless of whether or not clinicians have classified the disease as being bulbar in type (1, 2). The central question of whether these changes in RNA are of etiological importance in ALS has been discussed elsewhere (11). The cause of ALS remains elusive.

**REFERENCES**


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