Relation between RNA Content and Ageing in Neurons of the Dorsal Lateral Geniculate Nucleus

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ABSTRACT: We carried out a study to establish ribonucleic acid (RNA) content in the nucleus and cytoplasm of single neuronal cells from the dorsal lateral geniculate nucleus (dLGN) of 3–30 month-old rats. Mean RNA content was calculated as the product of RNA concentration and nuclear or cytoplasmic surface. The analysis of neuronal nuclei revealed no significant differences in RNA concentration, nuclear area, and RNA content from 3–18 months. However, a significant decrease in RNA concentration (18.73%) was found from the 18th–24th month, although no changes were observed in nuclear area and RNA content. The oldest rats, 24–30 months old, presented a significant increase in nuclear area and RNA content. As regards to the neuronal cytoplasm, no significant differences were found in any of the parameters at the ages from 3–18 months and 18–24 months. In contrast, a significant increase in RNA concentration (26.26%), cytoplasm area (18%), and RNA content (52%) takes place from the 24th–30th month. The increase in RNA content could be related to neuronal hypertrophy.

MATERIAL AND METHODS

Animals

Sixteen male albino Wistar rats were used in this study. They were subdivided into four groups according to their ages [i.e., 3, 18, 24, and 30 months (n = 4 per age group)]. The animals were provided with food and water ad lib and kept in a temperature-controlled room with a 12-h dark/light cycle. Their weight was between 275 ± 21 g at 3 months of age and 410 ± 30.8 g at 24 months of age.

Tissue Preparation

Animals were anesthetized with 8% chloral hydrate (0.1 ml/30 g weight). Fixation was achieved by intracardiac perfusion with buffered 10% formaldehyde (after a saline wash). The entire brains were then removed, and a block containing the dLGN dissected out and placed in the same fixative for 48 h. After this they were processed in the standard way for embedding in paraffin. Serial 8 μm coronal sections containing the dLGN were made through the block’s entire length and then stained with gallocyanine chromalum. Given the fact that neuronal population is postmitotic, we assume that the changes occurring in nucleic acid content corresponds to RNA, since DNA content would remain constant.

Cytophotometric Study

In order to quantify RNA content we used a Leitz MPV2 cytophotometer with a 581–18 nm interference filter. The analysis of neuronal nucleus and cytoplasm consisted in first calculating the mean extinction (nucleic acid concentration per surface unit, expressed in arbitrary units of light absorption) of 120 neurons per animal obtained by scanning the surface under analysis. Nucleus...
and cytoplasmic surface area (\(\mu m^2\)) was simultaneously calculated using drawings of their profiles traced with a camera lucida attached to the cytophotometer at a magnification of \(\times 100\). Profiles areas were then calculated using a Kontron MOP-AM2 image analysis system. Finally, nuclear and cytoplasmic RNA content, expressed in arbitrary units, was calculated as the product of the mean extinction and surface.

**Statistical Analysis**

Data are presented as mean ± standard error of the mean (SEM). A statistical analysis was first carried out using a computerised Kolmogorov-Smirnov test to evaluate whether or not the data followed a normal distribution. As this test rejected the hypothesis of a normal distribution for most parameters, the results obtained were compared using the Kruskal-Wallis test (\(p < 0.01\)).

**RESULTS**

**Nuclear Values**

From the 3rd–18th month the mean nuclear RNA concentration undergoes a nonsignificant increase of 12%. There is a significant decrease in the mean value from the 18th–24th month, from 0.2560 ± 0.0056 a.u. to 0.2156 ± 0.0049 a.u. (18.73%; \(p < 0.01\)). From the 24th–30th month no significant differences are observed (Table 1).

Study of the nuclear area reveals no significant change from the 3rd–24th month. However, from the 24th–30th month the nuclear area increases by 25.36% (\(p < 0.01\)).

Regarding total RNA content, no significant changes are found from either the 3rd–18th and 18th–24th month. Nevertheless, a statistically significant increase (34.92%; \(p < 0.01\)) was observed from the 24th–30th month.

**Cytoplasmic Values**

The mean cytoplasmic RNA concentration presents no significant change from the 3rd–18th month or the 18th–24th month. From the 24th–30th month, however, we find an increase of 26.26% (Table 1; \(p < 0.01\)).

No changes are found in the cytoplasmic area from the 3rd–24 month, but the mean area significantly increases from 62.99 ± 1.65 \(\mu m^2\) at the age of 24 months to 74.39 ± 1.97 \(\mu m^2\) at the age of 30 months (Table 1; \(p < 0.01\)).

RNA total content reveals no significant differences from the 3rd–24th month (Table 1), but it does show an increase from the ages of 24–30 months (52%; \(p < 0.01\)).

**DISCUSSION**

In spite of the universality of biological senescence, meaningful advances in the field of neurobiology are fairly recent. The main areas of research in this field have dealt with establishing which brain regions are more susceptible to ageing and defining the morphological, neurochemical, and physiological changes occurring during this process.

Worth mentioning among the many studies carried out are those trying to assess age-related histochemical changes in the CNS. Their relevance lies in the fact that these changes are correlated with a decrease in CNS function [26]. Among such changes we have elected to study changes in nucleic acid level in the nucleus and cytoplasm of neurons. We used gallocyanine-chromalum, which under carefully controlled conditions, can be used for estimating the amount of nucleic acid [8,19]. Some research exist of single neurons [24,25] that quantify nucleic acids. Recently, we have also studied nucleic acid content in neurons from the dorsocaudal region of the thalamic reticular nucleus during the ageing. We have observed that from the 24th–30th month (i.e., old age) nucleic acid per surface unit and total content in the cytoplasm exhibited a considerable decrease [18].

Our work is specifically focused on single dLGN neurons. In previous studies we have already determined several morphometric and histochemical parameters in the same region [6,29] such as size and shape of neurons and cytochrome oxidase activity.

The analysis of the neuronal nuclei consisted in first calculating RNA concentration per surface unit, a parameter expressed as the mean extinction. As approximately 85% of nuclear RNA is ribosomal RNA, 10% transfer RNA, and 1.5% messenger RNA [23], total RNA content mainly refers to the content in ribosomal subunits. Our study revealed no significant changes from the 3rd–18th month; however, it did show a 18.73% decrease at 24 months. From that point on no other significant changes were registered. We believe that this decrease at 24 months could reflect a drop in protein requirements and perhaps the first changes typical of the ageing process.

As with the nuclear area, the examination of total RNA content in the nucleus indicates that there are no changes until the 24th month. Nevertheless, from this age onwards we detected a significant increase in total nuclear RNA content reaching 24.26 ± 0.69 a.u. by the 30th month. Given that from the 24th–30th month there is nuclear hypertrophy, although RNA concentration per surface unit undergoes no modification, it is reasonable to deduce that the increase in total RNA content is solely due to an increase in nuclear size.

As regards neuronal cytoplasm we have found that from the 3rd–24th month RNA concentration per surface unit undergoes no

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Nuclear RNA Concentration</th>
<th>Cytoplasmic RNA Concentration</th>
<th>Surface</th>
<th>Nuclear</th>
<th>Cytoplasmic</th>
<th>RNA Content</th>
<th>Nuclear</th>
<th>Cytoplasmic</th>
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<tr>
<td></td>
<td>RNA Concentration</td>
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<td>RNA Concentration</td>
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<td></td>
<td>Nuclear</td>
<td>0.2286 ± 0.0058</td>
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<td>78.61 ± 1.79</td>
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<td>0.2050 ± 0.0046</td>
<td>80.87 ± 2.19</td>
<td>59.73 ± 1.66</td>
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<td>0.2156 ± 0.0049*</td>
<td>0.1843 ± 0.0042</td>
<td>85.03 ± 1.73</td>
<td>62.99 ± 1.65</td>
<td>17.98 ± 0.45</td>
<td>11.57 ± 0.39</td>
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<td>0.2351 ± 0.0070</td>
<td>0.2327 ± 0.0056**</td>
<td>106.60 ± 1.96**</td>
<td>74.39 ± 1.97**</td>
<td>24.26 ± 0.69**</td>
<td>17.58 ± 0.67**</td>
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*p < 0.01 as compared to 18–24 months old.

**p < 0.01 as compared to 24–30 months old.**

**TABLE 1**

RNA CONCENTRATION (A.U.), SURFACE (\(\mu m^2\)) AND RNA CONTENT (A.U.) IN SINGLE NEURONAL NUCLEI AND CYTOPLASM OF DORSAL LATERAL GENICULATE NUCLEUS AT DIFFERENT AGES. RESULTS ARE MEAN VALUES ± SEM.
significant changes, but from the 24th month a noticeable increase occurs. This increase in RNA concentration per surface unit could be related to the process of cell body hypertrophy taking place in the dLGN neurons during ageing. This has been calculated at 31% and 36% for the magnocellular and parvocellular layers in monkey dLGN [1] and at 22% in rat dLGN [29].

The RNA total content in the cytoplasm exhibited no change from 3–18 months and 18 –24 months. From the 24th–30th month a significant increase was found (11.57 ± 0.39 a.u. and 17.58 ± 0.67 a.u., respectively), which is mainly due to the increase in cytoplasmic area. Once again we believe that the increases found in cytoplasmic area as well as in total RNA content could be related to the process of cell body hypertrophy.

This hypertrophy could well be one of the first manifestations of senescence [3] because it would reflect the existence of a compensatory process (i.e., the increase of the dendritic tree to compensate for a possible reduction in the number of synapses or in their efficiency). This dendritic growth, with the consequent increase of the neuropil, could explain the increase in the total volume of the dLGN during senescence [1]. It is possible that at a subsequent chronological stage these compensatory adjustments of the neurons cease and, therefore, a decrease in neuronal size and in neuron synthesis organelles takes place. This would then lead to a cascade of events ending in neuronal dysfunction and death [12, 13].

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REFERENCES