Stability of brain RNA post mortem: effect of Alzheimer’s disease

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Many neurological and psychiatric disorders are genetic (e.g. Huntington’s chorea), have a genetic component (e.g. Alzheimer’s disease, bipolar depression) or are thought to involve alterations in gene expression (e.g. Pick’s disease, Alzheimer’s disease). It would, therefore, be useful to examine gene expression in post-mortem human brains. For this reason we have assessed the stability of brain RNA with post-mortem delay using sheep tissue. We have also isolated RNA from normal and Alzheimer human brain and determined its yield and integrity.

Total and mRNA was prepared from sheep brains at 0, 4, 8, 24 and 48 h post mortem. Before use the brains were stored so as to mimic post-mortem cooling conditions of human brain awaiting autopsy, and then frozen rapidly in liquid nitrogen. Total RNA was prepared by the guanidinium thiocyanate/CsCl method (Chirgwin et al., 1979) and mRNA by one passage of total RNA over an oligo(dT)-cellulose column. The amount of total RNA, as estimated by absorbance at 260 nm, remained unchanged up to 24 h post mortem and decreased to 50% of the fresh value from 24 to 48 h post mortem (Fig. 1a). The yield of mRNA expressed as a percentage of the total remained unaltered with post-mortem delay (Fig. 1b). Agarose gel electrophoresis and ethidium bromide staining of both total and mRNA showed no change in profile up to 48 h post mortem. The rate of in vitro protein synthesis of mRNA as measured by the incorporation of [3H]leucine was found to be similar for all mRNA samples of differing post-mortem delays (Fig. 1c). Autoradiographs of one-dimensional polyacrylamide gels indicated that high molecular mass proteins (at least up to 100 kDa) were synthesized (Laemmli, 1970; Marrota et al., 1981).

Total and mRNA was prepared from six normal human brains (post-mortem delay 7.3 ± 2.25 h, age 70 ± 6.4 years). The yield of total RNA was 167.5 ± 17.7 μg/g of starting tissue (slightly less than that of sheep). The percentage of mRNA obtained from total RNA (5.58 ± 1.2%) was within the normal range. This human RNA was electrophoresed in a denaturing gel containing 1.5% agarose/2.2 M-formaldehyde (Lehrach et al., 1977; Goldberg, 1980). The gel was blotted to hybond nylon (Amersham) (Southern, 1975) and fixed by U.V. irradiation for 5 min. The Northern blot was probed with a 32P-labelled cDNA actin probe (Minty et al., 1981). This probe detected a single band at 2 kb, indicating that the RNA is in an undegraded form. Agarose gel profiles of human RNA were similar to those of sheep. Translation of this human mRNA in vitro gave similar rates of protein synthesis to those found when using sheep brain.

To summarize, these data indicate that intact and biologically active mRNA can be isolated from post-mortem brain for at least 24 h post mortem. This suggests that it should be possible to study differential gene expression in health and disease. To this end total RNA and mRNA was prepared from six Alzheimer brains (age 70 ± 6.4 years, post-mortem delay 10 ± 3 hours). The yield of total RNA (137 ± 13 μg/g of starting tissue) was similar to that of the normal human brains and the yield of mRNA (3.3 ± 0.5%) was slightly less compared with the normal brains, but was within the normal range and similar to the results obtained using sheep tissue. These preliminary studies indicate that the yield of total RNA is the same in both control and Alzheimer brains, but suggests that the yield of mRNA is slightly lower in Alzheimer brain (Sajdel-Sulkowska et al., 1983, Taylor et al., 1986).

Fig. 1. Quantity and quality of sheep brain RNA at different post-mortem times

(a) Yield of total RNA (± S.E.M.), as estimated by A260nm, from sheep brain (n = 4). (b) Yield of sheep poly(A) RNA from total RNA (± S.E.M.) (n = 4). (c) Translation of mRNA in vitro (± S.E.M.), measured as the incorporation of [3H]leucine into protein (n = 4).


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Quantitative and qualitative characterization of serum and urine glycosaminoglycans in hyperthyroidism

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It is well established that thyroid hormones regulate the metabolism of virtually all mammalian tissues. During experimental application of thyroxine to animals and its therapeutic administration to man there is evidence of a fall in the levels of non-sulphated and sulphated glycosaminoglycans (GAG), compounds widely distributed in human tissues, mainly in connective tissue (Likar et al., 1982). Although the changes in serum and urine GAG in several endocrinopathies have been investigated, no information on the content and composition of serum and urine GAG in hyperthyroidism has been obtained (Likar et al., 1982).

Our study included 32 patients (female) with hyperthyroidism (average age 37 ± 8 years). Twenty reference samples were obtained from adult females (33 ± 7 years) free from acute illness and without any history of chronic disease. GAG were separated from serum by a modification of the method of Hata et al. (1978). Urine GAG were isolated using a method which was the combination of three previously known methods (Humbel, 1975; Taniguchi & Koizumi, 1975; Nagatsu et al., 1980). Serum and urine GAG were separated using two-dimensional electrophoresis on cellulose acetate membrane according to the method of Mossman & Patrick (1982). The strip was stained with Alcian Blue in methanol/acetic acid/water (50:5:45, by vol) for 15 min, destained with 5% acetic acid and further washed with water. Identification of electrophoretic spots was carried out using pure GAG standards. Further confirmation of GAG identification was carried out by treating of standards, serum and urine GAG with testicular hyaluronidase (Tani- guchi et al., 1974) and chondroitinases ABC and AC (Saito et al., 1968), and then electrophoresing the products using a standard assay procedure. Quantification of each individual GAG was carried out by the method of Hronowski & Anastasiades (1979). The estimation of isomeric chondroitin sulphates which were not accomplished electro- phoretically were achieved by measuring the borate-catalysed Morgan–Elson reaction colour of GAG samples according to the method of Saito et al. (1968). Uronic acid content was estimated by the carboxazole method of Bitter & Muir (1962).

The results of electrophoretic and enzymic analysis showed that hyperthyroidism patients and normal controls both contain chondroitin-4/6-sulphate as the main urine GAG fraction and heparan sulphate as the minor one. The main serum GAG in both groups studied was a low sulphated chondroitin-4-sulphate and the minor one was chondroitin-4-sulphate. The two-dimensional electrophoretic procedure we used allows separation and quantification of each GAG. Electrophoretic patterns of material obtained from hyperthyroidism patients were identical with normal control patterns; however, significant quantitative changes exist. The study of patients with hyperthyroidism shown the concentration of hexuronic acid GAG in urine to be 32% lower as compared with healthy subjects (Table I). A statistically significant decrease of heparan sulphate and chondroitin-4/6-sulphate level was observed (by 29% and 31% respectively). The average amount of GAG in the serum of patients with hyperthyroidism was increased by 51% compared with normal. This increase is due to the both serum GAG fractions: low sulphated chondroitin-4-sulphate and chondroitin-4-sulphate levels were elevated by 22% and by 190% respectively. The results obtained by the two methods used, the carboxazole method and determination after two dimensional electrophoretic separation, agreed well with each other.

In order to quantify chondroitin sulphate isomers the urinary and serum GAG were digested with chondroitinases and chondrosulfatases and the amount of chondroitin

Table 1. Total and fractional content of urinary and serum GAG in patients with hyperthyroidism and in healthy controls

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<thead>
<tr>
<th></th>
<th>Hyperthyroidism patients</th>
<th>Healthy controls</th>
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<tbody>
<tr>
<td><strong>Serum GAG content (μmol/l)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Total (measured by the carbazole method)</td>
<td>40.9 ± 7.9</td>
<td>26.9 ± 3.3</td>
</tr>
<tr>
<td>Low sulphated chondroitin-4-sulphate</td>
<td>30.3 ± 5.3</td>
<td>25.1 ± 3.3</td>
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<tr>
<td>Chondroitin-4-sulphate</td>
<td>15.0 ± 2.9</td>
<td>5.1 ± 0.9</td>
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*Values are expressed as uronic acid content.