Letters

Recovery and measurement of RNA in Alzheimer's disease by molecular hybridisation

Sir: RNA has been recovered from brains with post mortem delays of up to 16 hours with sufficient integrity to direct the in vitro synthesis of proteins. Thus techniques based on molecular hybridisation could also be used to quantify RNA recovery with the following advantages; they can detect lower abundance messenger RNA species than in vitro translation, the same samples can be rehybridised with several probes and it is not necessary that the RNA be intact to be detected, since fragments of a partially degraded molecule are still able to bind probes. We describe below the recovery and measurement by molecular hybridisation of ribosomal RNA and poly A containing messenger RNA from four cases of Alzheimer's disease and four neurologically normal control cases.

Cloned Drosophila melanogaster ribosomal cDNA was a generous gift from D Murphy, Imperial College, London. Tritiated poly uridylic acid (400 mCi/mmol) was from Amersham International Ltd. RNA was extracted from snap frozen tissue by powdering the tissue with solid carbon dioxide and homogenising in 6M guanidinium isothiocyanate, then layered over 5 M calcium chloride solution and centrifuged at 150,000g for 16 hours. The pellet was resuspended in water, and ethanol precipitated. For filter hybridisations with ribosomal cDNA, serial dilutions of denatured RNA were filtered in 20 x SSC (3 M NaCl, 0.3 M sodium citrate) under negative pressure through preretted nitrocellulose (Schleicher and Schuell BA 85) using a BRL hybridot manifold. The filters were allowed to dry then baked at 85°C for 2 hours. Molecular hybridisation was as described previously, based on the methods of Jeffreys, with the addition of polyuridylic acid at a concentration of 100 μg/ml in the prehybridisation, hybridisation and first wash buffers. The filters were dried and bound probe was quantified by liquid scintillation spectrophotometry. Solution hybridisation with 3H polyuridylic acid was as described by Bishop and Rosbash. RNA yields from human cortex were in the range 100-270 μg/g tissue based on OD260 measurements. Greater than 30% reductions in the mean levels of both ribosomal and poly A hybridisation signals were observed in the Alzheimer's disease group, but individual variation was greater in the control group (table).

Our findings show that measurable quantities of RNA can be recovered from human brains up to 72 hours post mortem. Classical histochemical methods have demonstrated a reduction in RNA levels in Alzheimer's disease brains, in addition to the reduction in total RNA extracted as measured by spectrophotometry. The hybridisation results with both ribosomal cDNA and poly U confirm these findings, showing a general reduction in the mean RNA levels in the frontal cortex of the Alzheimer's group compared with the controls.

The control group contained cases with widely differing causes of death, which may explain the greater variation in RNA levels recovered from the controls as measured by hybridisation compared with the Alzheimer's disease group. This means that not only will large numbers of samples have to be screened, but internal RNA standards will be needed to distinguish between disease specific changes and other sources of variation (for example cause or mode of death). The application of hybridisation techniques should enable both of these criteria to be met more easily than by in vitro translation and gel electrophoresis.

Table Ribosomal and poly A containing RNA in frontal cortex samples (Brodmann Area 46) from Alzheimer's disease

<table>
<thead>
<tr>
<th>Controls (SEM)</th>
<th>Alzheimer's disease (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pm delay (h)</td>
<td>20 (6-6)</td>
</tr>
<tr>
<td>age (yr)</td>
<td>78 (2-5)</td>
</tr>
<tr>
<td>ribosomal (kcpm)</td>
<td>5890 (766)</td>
</tr>
<tr>
<td>poly U (kcpm)</td>
<td>15440 (2093)</td>
</tr>
</tbody>
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