tation, we have monitored the binding of the muscarinic agonist oxotremorine-M in the presence of Mg\(^{2+}\) ions and the non-hydrolysable GTP analogue guanylylimidodiphosphate (p[NH)pG]. Having established the stability of these interactions with regard to freezing and autopsy delay, in mouse cortex, we report here our findings in parietal cortex from a series of Alzheimer and control cases with short post-mortem delay intervals; parietal cortex being an area primarily affected in Alzheimer’s disease.

Tissue was sampled from Brodmann area 39/40 for each case and grey matter, through all cortical levels, was carefully dissected from white matter (coronal blocks had been previously frozen in liquid arctic and kept stored at \(-70^\circ\)C). The tissue was then homogenized on ice in 10 vol. (w/v) of 20 mm-sodium Hepes buffer, pH 7.6, with 10 mm-EDTA added. The homogenate was centrifuged at 26 000 g for 20 min at 4°C and the pelleted membranes were washed twice using buffer containing 1 mm EDTA. Triplicate 1 ml membrane suspensions, each containing 5 mg of original wet weight of tissue in 20 mm-sodium Hepes (no EDTA), were incubated at 25°C for 20 min with 1 mm-[\(^3\)H]oxotremorine-M (NEN, sp. act. 87.0 Ci/mmol) alone or with the following additions: (1) 1 mm-MgCl\(_2\); (2) 1 mm-MgCl\(_2\) + 100 \(\mu\)m p[NH)pG; (3) 1 \(\mu\)m-atropine to define specific muscarinic binding.

Assays were terminated by rapid filtration using a semi-automatic Skatron cell harvester and Whatmann GF/B filters. The homogenate was centrifuged at 26 000 g for 20 min at 4°C, and the pelleted membranes were washed twice using buffer containing 1 mm EDTA.Triplicate 1 ml membrane suspensions, each containing 5 mg of original wet weight of tissue in 20 mm-sodium Hepes (no EDTA), were incubated at 25°C for 20 min with 1 mm-[\(^3\)H]oxotremorine-M (NEN, sp. act. 87.0 Ci/mmol) alone or with the following additions: (1) 1 mm-MgCl\(_2\); (2) 1 mm-MgCl\(_2\) + 100 \(\mu\)m p[NH)pG; (3) 1 \(\mu\)m-atropine to define specific muscarinic binding.

The results are shown in Table 1. There was a significant decrease in the reversible formation of high-affinity receptor states in Alzheimer’s disease (30%), reflected by an overall reduction in the binding of oxotremorine-M in the presence of Mg\(^{2+}\) and in the proportion of H sites which could be modulated by p[NH)pG. There were no correlations between any of the measured parameters and age or post-mortem delay in either the control or diseased group.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Post-mortem delay (h)</th>
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<tbody>
<tr>
<td>74 ± 12</td>
<td>14 ± 17</td>
</tr>
<tr>
<td>77 ± 11</td>
<td>16 ± 13</td>
</tr>
</tbody>
</table>

Table 1. Modulation of [\(^3\)H]oxotremorine-M binding in control and Alzheimer’s disease parietal cortex

All values given as mean ± s.e.m. Units for [\(^3\)H]oxotremorine-M binding are fmol/mg of protein. *P < 0.05, using Student’s t-test.

Synthesis of [\(^14\)C]-labelled \(\alpha\)-amino-\(\beta\)-methylaminopropionic acid and its metabolism in the rat

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\(\alpha\)-Amino-\(\beta\)-methylaminopropionic acid (synonym \(\beta\)-amino-\(\alpha\)-alanine; \(\alpha\)-BMAA) is a convulsant in small experimental animals (Polsky et al., 1972), a stereospecific neurotoxin in foetal mouse spinal cord cultures (Nunn et al., 1987) and generates a motor systems dysfunction, with Parkinsonian features, when fed to macaques (Polsky et al., 1972), since its neuronototoxicity in these preparations is attenuated by the NMDA receptor antagonist 2-amino-7-phosphonoheptanate.

Since \(\alpha\)-BMAA is a neutral amino acid at physiological pH, it is evident that it does not itself activate NMDA receptors. However, BMAA may act via a number of indirect mechanisms in which it (a) is converted to a metabolite that acts at the NMDA receptor, (b) inhibits a reaction that causes the accumulation of an NMDA receptor activator, or (c) stimulates a metabolic pathway that allows the accumulation of a metabolic active at the NMDA receptor. We are investigating the possibilities encapsulated in (a) with the aid of \(\alpha\)-BMAA labelled with \(^14\)C in the methyl group.

The labelled \(\alpha\)-BMAA (specific activity 41 \(\mu\)Ci/mmol) was prepared by an adaptation of an existing method (Vega et al., 1968). Its authenticity and purity were demonstrated by elemental analysis, mass spectroscopy, proton n.m.r., high-voltage electrophoresis (aqueous pyridine/acetate acid, pH 3.6) and t.l.c. (butanol/acetate acid/water, 1:1:1, by vol.). The radioactive material was administered to male rats (111-140 g) fed Purina Chow and water ad libitum while...
housed in individual metabolic cages. The animals were injected intraperitoneally daily with unlabelled 1-t-BMAA for 6 days [0.28 mg (1.8 μmol)/g per day]. From the seventh day onward, 1 μCi of the labelled material was also injected. Urine from rats thus treated was collected in thymol and stored at −70°C until appropriate samples were deproteinized before analysis by adding an equal volume of cold 10% (w/v) sulphosalicylic acid, followed by centrifugation for 3 min at 4°C in a microcentrifuge. The supernatant was added to an equal volume of 1 M-HCl and inserted into the loading ports of a Locarte aminoacid analyser (using a standard Li+-buffer system with an elution rate of 70 ml/h); Fractions (1.5 min) of the eluate were collected and mixed with an equal volume of PCS (Amersham). Radioactivity was detected by liquid scintillation counting.

Using this analytical system the 14C-labelled 1-t-BMAA yielded at least six radioactive peaks. As a result of this metabolic diversity we endeavoured to gain more knowledge of the eluate were collected and mixed with an equal volume of

salicylic acid, followed by centrifugation for 3 min at 4°C in a microcentrifuge. The supernatant was added to an equal volume of 1 M-HCl and inserted into the loading ports of a Locarte aminoacid analyser (using a standard Li+-buffer system with an elution rate of 70 ml/h). Fractions (1.5 min) of the eluate were collected and mixed with an equal volume of

PCS (Amersham). Radioactivity was detected by liquid scintillation counting.

Direct evidence to suggest that AlF4⁻ is not the active species involved in fluoride/aluminium G-protein interaction

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Micromolar concentrations of aluminium have been shown to facilitate the fluoride stimulation of a variety of G-proteins (Sternweis & Gilman, 1982; Blackmore et al., 1985; Paris & Pouyssegur, 1987; Kanako et al., 1985). It has been suggested that this is due to the formation of AlF4⁻, that activates the GTP–GDP complex by mimicking the γ-phosphate of GTP in its binding site (Bigay et al., 1985; 1987). However, we are not aware that any attempt has been made to test this directly. Hence we have synthesized NaAlF4 and studied its effect on cyclic AMP formation and phosphoinositide (PI) hydrolysis in isolated rat cerebral cortex.

Crystalline NaNAlF4·H2O was synthesized from AlF3 and NaF by the method of Grobelyn (1976), and its composition was confirmed by X-ray diffraction. The aluminium content of buffers was determined by atomic absorption spectroscopy. No glass vessels were used in this work and dust contamination was avoided as far as possible. The activation of adenylate cyclase was studied in suspensions of washed membranes. Tissue was homogenized in 10 vol. of ice-cold buffer containing 10 mm-Tris/maleate pH 7.4, 2 mm-EGTA and 1 mm-dithiothreitol and centrifuged for 9000 g for 3 min at 4°C. The precipitate was washed 3 times in the same buffer. Aliquots (50 μl) of membrane suspension containing approximately 200 μg of protein were incubated for 5 min at 37°C in 450 μl of buffer composed of 82.5 mm-Tris/maleate pH 7.4, 20 mm-MgSO4, 0.5 mm-EGTA, 1 mm-3-isobutyl-1-
methyloxanthine, 0.05 mm-GDP and 5 mm-ATP, with and without additions (see Table I). The incubation buffer was found to contain 3.7 μM-aluminium, 50% of this contamination resulting from the ATP. Experiments were carried out in triplicate and the reaction was stopped by incubation at 90°C for 5 min. Samples were centrifuged at 9000 g for 3 min, the supernatants stored at −20°C and cyclic AMP was assayed according to Brown et al. (1971). PI hydrolysis in cortical miniprisms was determined by the method of Court et al. (1986). Aluminium contamination of the modified Krebs–Henseleit bicarbonate used was less than 0.2 μM.

AlF3 failed to stimulate either cyclic AMP production at 1, 10 or 100 μM or PI hydrolysis at 10 μM, whereas 3 μM-AlF3 in the presence of 10 μM-F⁻ stimulated both second messenger systems (Table I). In addition, forskolin stimulated adenylate cyclase in cortical membranes and carbachol stimulated phospholipase C in cortical miniprisms (significantly at 16 mm-K⁺). The presence of raised K⁺ did not appear to facilitate aluminium stimulation of the PI response, although this was the case with carbachol (Table I).

There are several possible explanations for the lack of effect of AlF3. First it is relatively insoluble; however, the aluminium content of stock solutions of AlF4⁻ (3.5 and 0.35 mm) was confirmed by atomic absorption spectroscopy. Secondly, the possible dissociation of AlF3, and in aqueous solution (3.5 mm) 19F-n.m.r. spectroscopy showed approximately equimolar amounts of AlF4⁻, AlF3, and F⁻; thus some dissociation of the AlF4⁻ anion does occur in solution. Finally, in the experiments using miniprisms, it is possible that the AlF3 ion was unable to permeate the tissue; however, this should not have been the case with the membrane homogenates.

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Vega, A., Bell, E. A. & Nunn, P. B. (1968) Physicochemistry 7, 1885–1887