Preservation of acetylcholine muscarinic M2 receptor G-protein interactions in the neocortex of patients with Alzheimer’s disease

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Abstract

The efficacy of acetylcholine muscarinic M2 receptor–G-protein coupling was investigated in Alzheimer’s disease and control neocortical membranes by measuring the effects of MgCl2 and 5’-guanylylimidodiphosphate (Gpp[NH]p) on high-affinity [3H]oxotremorine-M ([3H]OXO-M) binding. MgCl2 gave similar enhancements of [3H]OXO-M binding in Alzheimer’s disease and control occipital cortex. In contrast, MgCl2 enhanced [3H]OXO-M binding was significantly higher in Alzheimer’s disease superior temporal cortex, compared to controls. MgCl2 enhanced [3H]OXO-M binding in both the occipital and temporal cortices of the Alzheimer’s disease cases was reversed to control levels by Gpp[NH]p. It is concluded that the number of high-affinity muscarinic M2 sites is increased in Alzheimer’s disease superior temporal, but not occipital, cortex and that M2 sites in both regions maintain an efficient G-protein coupling.

Keywords: Alzheimer’s disease; G-protein; Post-mortem human brain; Acetylcholine; Muscarinic receptor

Despite convincing evidence that the early degeneration of ascending cholinergic neurons contributes to Alzheimer’s disease dementia [24], most attempts to ameliorate the cognitive decline in the disorder by enhancing cholinergic neurotransmission have proven disappointing [24]. We and others have hypothesised that the lack of success of cholinergic treatment strategies for Alzheimer’s dementia may result in part from inefficient coupling of target acetylcholine muscarinic receptor sites to their effector mechanisms [5,6].

Acetylcholine muscarinic receptors comprise a family of five separate gene products, termed m1–m5, whose effects are mediated by G-proteins. The m2 and m4 gene products are coupled via pertussis toxin-sensitive G-proteins to the inhibition of adenyly cyclase [15] whereas the m1, m3 and m5 receptor effects are mediated mainly by pertussis toxin-insensitive G-proteins and include stimulation of phospholipases C, D and A2 [2,15,16]. The m1, m2 and m3 gene products correspond, respectively, to the M1, M2 and M3 receptor types, that have been defined on the basis of antagonist selectivity.

Evidence for disrupted acetylcholine muscarinic receptor–G-protein interactions in Alzheimer’s disease brain has come from a number of studies. By measuring the effects of MgCl2 and 5’-guanylylimidodiphosphate (Gpp[NH]p) on carbachol agonist displacement of [3H]pirenzepine antagonist binding, Smith et al. were able to show an attenuation of M1 receptor–G-protein interactions in Alzheimer’s disease parietal cortex [19]. Using a similar assay, Flynn et al., reported a loss of high-affinity agonist binding to M1 receptors in Alzheimer’s disease frontal cortex [5]. Recently, the same group reported that this loss of high-affinity M1 sites correlated with a decreased ability of the muscarinic agonist carbachol to stimulate GTP-dependent [3H]phosphatidylinositol-4,5-bisphosphate hydrolysis, indicating a defective muscarinic receptor–G-protein/phospholipase C coupling in the disease [4]. Muscarinic receptor–G-protein interac-
tions have also been suggested to be disrupted in Alzheimer's disease thalamus [23], whereas others have claimed that M1 receptor coupling is preserved in the mid-temporal cortex [13].

The integrity of G-protein coupling to muscarinic receptors other than the M1 subtype has not been extensively studied in Alzheimer's disease. In a preliminary study, Smith et al. reported a small decrease in the number of high-affinity, G-protein mediated, muscarinic M2 sites in Alzheimer's disease parietal cortex, as determined by measuring the effects of MgCl$_2$ and Gpp[NH]p on $[^3H]$oxotremorine-M ($[^3H]$OXO-M) agonist binding [20]. In the present study, we performed similar $[^3H]$OXO-M binding assays to determine the efficiency of muscarinic M2 receptor G-protein coupling in Alzheimer's disease superior temporal and occipital cortices. These regions were chosen as typically showing severe and mild Alzheimer's disease pathology, respectively [14].

Tissue for these studies was dissected from the superior temporal (gyrus temporals superior) and occipital (gyrus occipitalis) cortices from a group of seven controls and Alzheimer's disease cases. Tissue was dissected fresh, frozen on liquid nitrogen and stored at -80°C. Clinical diagnoses of Alzheimer's disease cases, respectively, were 7.66 ± 0.76 and 11.29 ± 2.02 (P < 0.01) and F$_{5.65}$ = 20.96 (P < 0.001). F$_{5.77}$ = 0.81 (NS) and F$_{5.65}$ = 0.09 (NS) for occipital cortex.

$[^3H]$OXO-M binding was significantly enhanced by MgCl$_2$ in a dose-dependent manner in both brain regions of the control and Alzheimer's disease cases (Fig. 1). However, in the superior temporal cortex, it was seen that MgCl$_2$ gave a greater enhancement of $[^3H]$OXO-M binding in the Alzheimer's disease cases, compared to controls (Fig. 1A). The mean (fmol/mg protein ± SEM) values for $[^3H]$OXO-M binding in the control and Alzheimer's disease cases, respectively, were 2.67 ± 0.70 and 3.56 ± 0.67 for the superior temporal cortex and 2.32 ± 0.24 and 1.77 ± 0.76 for the occipital cortex.

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Fig. 1. Effect of MgCl$_2$ on $[^3H]$oxotremorine binding to EDTA washed control and Alzheimer's disease superior temporal and occipital cortex membranes. Data represent the means ± SEM from 7 controls (open circles) and 6 Alzheimer's disease (closed circles) cases for the temporal cortex (A) and 6 controls (open circles) and 5 Alzheimer's disease (closed circles) cases for the occipital cortex (B). Two-way ANOVA gave values for the effects of MgCl$_2$ on $[^3H]$OXO-M (MgCl$_2$ and 0.001–10 mM) and Gpp[NH]p (0.01–100 mM, final concentration) on the concentration of MgCl$_2$ (0.001–10 mM, final concentration). Incubations were performed with constant shaking for 20 min and terminated by vacuum filtration onto Whatman GF/B glass fibre filters that had been pre-soaked overnight in 0.05% polyethyleneimine. Filters were washed rapidly three times with 5 ml of assay buffer and allowed to dry at room temperature. Filter bound radioactivity was determined by scintillation spectroscopy. All assays were performed in triplicate with non-specific binding defined using 1 µM atropine. Membrane protein content was determined using a modification of the standard Lowry method [8] and results expressed as fmol bound/mg protein.

The concentration (1 nM) of $[^3H]$OXO-M used in these studies was that reported to label exclusively the high-affinity agonist state of the M2 receptor [7,20].

**P < 0.01. +0.10 < P < 0.05.**
Fig. 2. Effect of Gpp(NH)p on magnesium enhanced [3H]oxotremorine binding to EDTA washed control and Alzheimer’s disease temporal and occipital cortex membranes. Data represent the means ± SEM from 7 controls (open circles) and 6 Alzheimer’s disease (closed circles) cases for the temporal cortex (A) and 6 controls (open circles) and 5 Alzheimer’s disease (closed circles) cases for the occipital cortex (B). Two-way ANOVA gave values for the effects of Gpp(NH)p, diagnosis, and the interaction diagnosis × Gpp(NH)p, respectively, of $F_{5,77} = 18.60$ ($P < 0.001$), $F_{1,77} = 4.11$ ($P < 0.05$) and $F_{5,77} = 1.63$ (NS) for temporal cortex and $F_{5,65} = 13.97$ ($P < 0.001$), $F_{1,65} = 2.75$ (NS) and $F_{5,65} = 0.03$ (NS) for occipital cortex. Significance levels shown are from Fisher’s post-hoc t-tests. **$P < 0.01$, *$P < 0.05$.

The results are in accordance with the data of Smith et al. who, using similar assay conditions, reported a significant 30% reduction of 1 mM MgCl₂ enhanced [3H]OXO-M binding in Alzheimer’s disease temporal cortex compared to levels seen in the control cases (Fig. 2A). This result indicates that the additional high-affinity muscarinic M2 sites seen in the Alzheimer’s disease temporal cortex show an efficient G-protein coupling. Fig. 2B shows that the profiles of Gpp(NH)p inhibition of MgCl₂ enhanced [3H]OXO-M binding were not significantly different in the occipital cortex of the Alzheimer’s disease and control cases, indicating that muscarinic M2 sites in this region also show a similar G protein coupling efficiency. These results are in accordance with the data of Smith et al. who showed that [3H]OXO-M binding in the presence of both 1 mM MgCl₂ and 100 μM Gpp[NH]p was not significantly different in Alzheimer’s disease and control parietal cortex [20].

Spearman’s rank correlation analyses of the data did not reveal any significant effects of age, postmortem delay and tissue pH on [3H]OXO-M binding, determined in either the absence or presence of MgCl₂ (1 mM) or in the presence of both MgCl₂ (1 mM) and Gpp[NH]p (10 μM). The lack of effect of age and postmortem delay on these [3H]OXO-M binding parameters has also been reported by Smith et al. for the parietal cortex [20]. It is therefore unlikely that the results of the present study reflect differences in the matching of samples between the control and Alzheimer’s disease groups.

Recently, Wang and Friedman demonstrated that the ability of carbachol to stimulate [35S]GTPγS binding to the G protein G₁₃ and G₁₃α-subunit isoforms was significantly decreased in Alzheimer’s disease frontal cortex, compared to controls [22]. Given that the receptor product of the muscarinic m2 gene has been demonstrated to couple potently to the G₁₃, G₁₂ and G₃α-subunit isoforms [10], it could be argued that the data of Wang and Friedman reflects an uncoupling of acetylcholine muscarinic M2 receptors from their respective G proteins in Alzheimer’s disease. This conclusion would contradict the data of the present study and that of Smith et al which show that the efficiency of acetylcholine muscarinic M2 receptor site–G-protein coupling is preserved in Alzheimer’s disease neocortex. However, the muscarinic m1 and m3 gene products have also been demonstrated to couple to G₁₁ and G₁₃α-subunits [10]. Thus, the data of Wang and Friedman may in part reflect the uncoupling of muscarinic M1 receptors from their G-proteins in Alzheimer’s disease [5,19,23].

The preservation of acetylcholine muscarinic M2 receptor site–G-protein interactions in Alzheimer’s disease neocortex is consistent with those studies showing that other neurotransmitter receptors that mediate the inhibition of adenylyl cyclase, including the α₂ adrenoceptor [12], serotonin 5-HT₁A [11], somatostatin [1,18] and adenosine A₁ [21] types, also show a maintained G-protein coupling in the disorder. This, together with a study showing that adenosine A₁ receptor [17] and G₃-
protein [3] mediated inhibition of adenyl cyclase activity is also preserved in Alzheimer’s disease neocortex indicates that the inhibitory receptor/Gi-protein regulation of this enzyme remains intact in the disease for a range of neurotransmitter receptor types [3,17].

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