Palmitoylation of metabotropic glutamate receptor subtype 4 but not la expressed in permanently transfected BHK cells.

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It has recently been shown that a number of G-protein coupled receptors, namely rhodopsin, the b2-adrenergic receptor, the a2-adrenergic receptor, the 5HT1B receptor and the D1 dopamine receptor are post-translationally modified by the covalent attachment of palmitic acid to one or more cysteine residues, via a hydroxylamine labile thioester bond [1-7]. In the case of the b2-adrenergic receptor, which is a Gq4 linked receptor, a rapid increase in the level of receptor palmitoylation has been observed in the presence of agonist [2]. Also, removal of palmitate by mutation of the site of attachment Cys 341 to glycine, resulted in a receptor that was functionally uncoupled from Gq4 and highly phosphorylated [3-4]. It has therefore been suggested that rapid agonist mediated turnover of receptor bound palmitate may be involved in regulating phosphorylation and desensitization of this receptor.[4]. In contrast, mutation of the site of palmitoylation in the Gq4 linked b2-adrenergic receptor, resulted in a receptor that was not palmitoylated but which coupled normally to Gq4, arguing against a role for palmitoylation in the regulation of signal transduction in this case [5].

The metabotropic glutamate receptors (mGluRs) form a distinct family of G-protein coupled receptors with seven putative membrane spanning domains. They are distinctive in that they exhibit no sequence homology with and are generally much larger than the other G-protein coupled receptors [8]. To date seven receptor subtypes have been cloned (mGlur1-7), with differing pharmacological properties and tissue distribution [8-14]. Two of these subtypes, mGlur1a, which is coupled to phospholipase C, and mGlur4, which is coupled to inhibition of adenyl cyclase, were examined for the possibility that they are too palmitoylated.

BHK cells permanently transfected with mGlur1a and mGlur4 respectively, were isotopically labelled with [3H]palmitic acid, but as controls the palmitoylated, endogenously expressed G-protein subunits a and a11 could readily be immunoprecipitated from the same cell lysates. These results therefore suggest that mGlur1a is not detectably palmitoylated.

In contrast, repetition of these experiments with BHK cells expressing mGlur4 clearly demonstrated incorporation of [3H]palmitate into this receptor, and the [3H]radiolabel was quantitatively removed by treatment with 1M hydroxylamine (pH 7.4) (Fig. 2.). This data therefore provides the first evidence that mGlur4 is modified by thioester linked palmitate. Agonist stimulation of the receptor in these cells with maximal doses of glutamate (1mM) for periods of 1, 5 and 10 minutes appeared to have no effect on the levels of receptor palmitoylation. This argues against a link between palmitoylation of this Gai linked receptor and the regulation of signal transduction, as in the case of the similarly Gq4 linked a2-adrenergic receptor [5].

These results demonstrate that of the two receptor subtypes studied, mGlur4, which couples to adenylyl cyclase, was found to be modified by palmitate, whereas mGlur1a, which couples to phospholipase C, was not detectably palmitoylated in our cells. This is consistent with the fact that to date all the G-protein coupled receptors that have been shown to be palmitoylated, except for the retinal receptor rhodopsin, are coupled to adenylyl cyclase and none have been found which couple to phospholipase C [1-7].

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Fig. 1. Immunoprecipitation of Tran [35S] mGlur1a and mGlur4

Fig. 2. Palmitoylation of mGlur4