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Down-regulation of phospholipase C-β_1 following chronic muscarinic receptor activation

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Abstract

To determine whether prolonged activation of a phospholipase C-coupled receptor can lead to a down-regulation of its effector enzyme, SH-SY5Y neuroblastoma cells were incubated for 24 h with the muscarinic receptor agonist, oxotremorine-M. Under these conditions, significant reductions (46–53%) in muscarinic cholinergic receptor density, $G_{\alpha q/11}$ and phospholipase C-β_1 (but not the β_3-or γ_1 isoforms) were observed. These results suggest that a selective down-regulation of phospholipase C-β_1 may play a role in adaptation to chronic muscarinic receptor activation. © 1998 Elsevier Science B.V.

Keywords: G protein-coupled receptor; Phosphoinositide-specific phospholipase C; Oxotremorine-M

Agonist occupancy of G protein-coupled receptors can initiate rapid physiological responses. However, prolonged exposure (h) to an agonist often results in a subsequent diminution of these responses due, in part, to a reduction in total receptor number (down-regulation). More recent evidence has demonstrated that in addition to the loss of receptors, prolonged agonist occupancy of G protein-coupled receptors can elicit a selective reduction in the concentration of the relevant G protein (Milligan, 1993). However, whether the effector enzyme is subject to down-regulation following prolonged receptor activation remains unknown. In this study we report that chronic agonist occupancy of muscarinic cholinergic receptors results not only in a down-regulation of both the receptor and $G_{\alpha q/11}$, but also in a selective loss of the β_1 isoform of phospholipase C.

In the present investigation, we utilized human SH-SY5Y neuroblastoma cells which possess a high density of m_3 muscarinic cholinergic receptors that couple to the $G_{q/11}$-mediated (pertussis toxin-insensitive) activation of phospholipase C-β_1. Cells were treated with 1 mM oxotremorine-M, a muscarinic receptor agonist, in Dulbecco's modified Eagle's medium/10% fetal calf serum for 24 h at 37°C (10% CO_2). Western blot analysis was utilized to determine the expression of isoforms of phospholipase C and G_α proteins in lysates as previously described (Sorensen et al., 1997). Incubation of cells with oxotremorine-M resulted in a marked reduction in the immunoreactivity associated with phospholipase C-β_1, the primary effector of muscarinic cholinergic receptor activation (Fig. 1A). Quantitation of the Western blots by densitometric analysis revealed that exposure of cells to the agonist resulted in a $46 \pm 9\%$ reduction in the level of phospholipase C-β_1 ($n = 7$, $P < 0.01$, Fig. 1C). This reduction was selective since no change in the immunoreactivities associated with either phospholipase C-β_3 (which is primarily activated by $G_{\beta\gamma}$) or phospholipase C-γ_1 (which is coupled to receptor tyrosine kinase activation) was observed (Fig. 1A,C). A similar reduction in phospholipase C-β_1 immunoreactivity was detectable after 4 h of agonist treatment (data not shown). Oxotremorine-M also induced a selective reduction ($53 \pm 10\%$, $n = 3$, $P < 0.05$) in the level of $G_{\alpha q/11}$ in the absence of any significant effect on $G_{\alpha s}$ (Fig. 1B,C). Determination of muscarinic cholinergic receptor expression, as monitored by [^{3}H]quinuclidinyl benzilate binding (Sorensen et al., 1997), revealed an agonist-induced 47 ±

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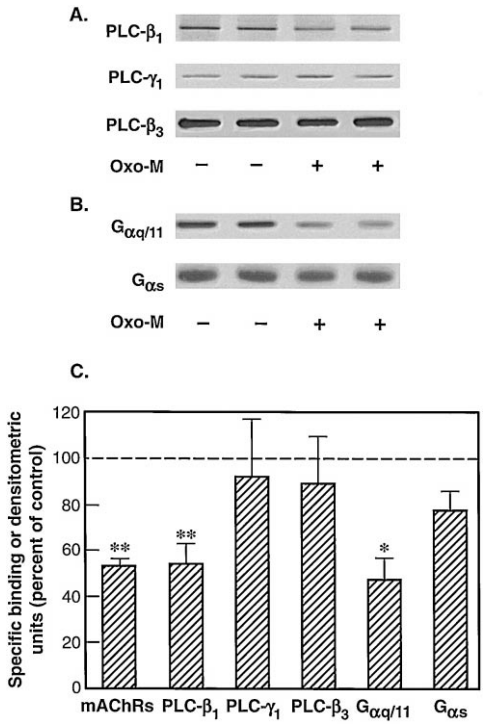

Fig. 1. Effect of chronic muscarinic receptor agonist treatment on cellular expression of phospholipase C (PLC) isoforms, $G_{\alpha q/11}$, $G_{\alpha s}$ and muscarinic cholinergic receptors (mAChRs). Cells were incubated with oxotremorine-M (Oxo-M) for 24 h as stated in the text. Equivalent aliquots (25 μg) of cellular protein from cell lysates were electrophoresed through 10% sodium dodecyl sulfate–polyacrylamide gels and transferred to polyvinylidene fluoride membranes. Membranes were immunoblotted for PLC-β_1, PLC-β_3, or PLC-γ_1 in (A) and for the α subunits of $G_{q/11}$ or G_s in (B). Panel (C) depicts the results of densitometric analysis of the Western blots and changes in [^{3}H]quinuclidinyl benzilate binding (to detect total cellular mAChRs) in whole-cell assays. Values are expressed either as specific binding or densitometric units relative to control cells (vehicle alone). The results shown are means ± S.E.M. of three ($G_{\alpha q/11}$ and $G_{\alpha s}$) or seven (PLC isoforms, mAChRs) separate experiments, each performed in duplicate or triplicate. * Different from control, $P < 0.05$; ** $P < 0.01$.

3% loss of receptor binding sites ($n = 7$, $P < 0.01$, Fig. 1C), a value similar to that observed for either the down-regulation of phospholipase C-β_1 or $G_{\alpha q/11}$.

Activation of either thrombin or collagen receptors on platelets has been shown to induce a calpain-dependent cleavage of the carboxyl terminal region of phospholipase C-β_3. However, the truncated product retains enzyme activity and can still be activated by $\beta\gamma$ subunits (Banno et al., 1995). In the present study, breakdown products of phospholipase C-β_1 were not detectable by an antibody which recognizes the carboxyl terminal portion of the enzyme. Moreover, calpain-mediated cleavage of phospholipase C-β_1 results in a form of the enzyme that is refractory to activation by $G_{\alpha q}$ (Park et al., 1993). To the best of our knowledge, the present results are the first to demonstrate that chronic activation of a phospholipase C-coupled receptor elicits not only the down-regulation of the cell-surface receptor and its corresponding G protein, but also of the specific isoform of phospholipase C to which the receptor is linked. For many signaling pathways, a reserve of receptor and G protein exists, and thus the effector enzyme is more likely to be the rate-limiting factor; therefore regulation at the level of the effector enzyme may have a profound effect on the cell's ability to respond to a specific receptor agonist. Furthermore, the down-regulation of phospholipase C could be of functional importance in terms of the heterologous regulation of other signaling pathways elicited following the activation of a phosphoinositide-linked receptor (for review, see Fisher, 1995). In conclusion, the present study demonstrates the selective down-regulation of phospholipase C-β_1 in response to muscarinic cholinergic receptor activation. This regulatory event is likely to serve as a key mechanism in attenuating agonist-driven cellular responses.

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