Phosphodiesterase-4 gates the ability of protein kinase A to phosphorylate G-protein receptor kinase-2 and influence its translocation

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Abstract

Challenge of the β2Ar (β2-adrenergic receptor) with isoprenaline in HEK-293β2 cells (human embryonic kidney cells stably overexpressing a FLAG- and green fluorescent protein-tagged β2Ar) results in the PKA (cAMP-dependent protein kinase) phosphorylation of GRK2 (G-protein receptor kinase-2). This response was enhanced when PDE4 (phosphodiesterase-4) activity was attenuated using either rolipram, a PDE4-selective inhibitor, or with siRNA (small interfering RNA) knockdown of both PDE4B and PDE4D. Rolipram also facilitated GRK2 recruitment to the membrane and phosphorylation of the β2Ar by GRK2 in response to isoprenaline challenge of cells. In resting cells, rolipram treatment alone is sufficient to promote PKA phosphorylation of GRK2, with consequential effects on GRK2 translocation and GRK2 phosphorylation of the β2Ar. Similar effects are observed in cardiac myocytes. We propose that PDE4 activity protects GRK2 from inappropriate phosphorylation by PKA in resting cells that might have occurred through fluctuations in basal cAMP levels. Thus PDE4 gates the action of PKA to phosphorylate GRK2.

Introduction

GPCRs (G-protein-coupled receptors) mediate cellular responses to a plethora of environmental stimuli through the activation of heterotrimeric G-proteins [1]. GPCR stimulation initiates a variety of intracellular signals, including classical second-messenger pathways such as the cAMP signalling cascade. PDEs (phosphodiesterases) play a pivotal role in signal transduction as they are the sole agents of cAMP degradation in cells [2].

The PDE4 family of cAMP-specific PDEs has been shown to have an important role in the compartmentalization of cAMP signal generation owing to unique N-terminal regions that target these enzymes to specific intracellular locations or signalling complexes. A novel facet of PDE4 function was uncovered when it was discovered that PDE4s, and PDE4D5 in particular, could bind to and translocate with the multifunctional scaffold protein β-arrestin [3,4]. The primary function of β-arrestins is to desensitize activation of GPCRs by physically blocking the signal between activated receptors and coupled G-proteins. Translocation of β-arrestin proteins to activated GPCRs is triggered by GRK (G-protein receptor kinase) phosphorylation of receptor intracellular domains [5]. Recruitment of PDE4–β-arrestin complexes to the β2Ar (β2-adrenergic receptor) results in a previously unrecognized dual-desensitization event whereby β-arrestin hinders Gs signalling and down-regulates cAMP production by adenylate cyclase while concomitant hydrolysis of localized cAMP by recruited PDE4 effectively resets the system for another round of agonist challenge [6]. In the present paper, we report that PDE4 activity can also influence one of the initial events in β2Ar desensitization, namely the translocation of GRK2 to activated GPCRs.

Inhibition of PDE4 activity enhances PKA (cAMP-dependent protein kinase) phosphorylation of GRK2

The activity and function of GRKs has been shown to be regulated by a number of different mechanisms, which include phosphorylation by other kinases. For instance, it is known that PKA activated by Gs-coupled receptors can phosphorylate GRK2 directly, enhancing binding to Gβγ subunits to facilitate membrane targeting [7]. In HEK-293β2 cells [human embryonic kidney cells stably expressing the FLAG- and GFP (green fluorescent protein)-tagged β2Ar], we observed a time-dependent isoprenaline-induced PKA phosphorylation of GRK2, which was ablated by the PDE4-specific inhibitor rolipram. Pre-treatment with the PDE4-specific inhibitor rolipram not only accelerated the ability of isoprenaline to cause the PKA phosphorylation of GRK2, but also, to a lesser degree, was able to initiate PKA phosphorylation of GRK2 under basal conditions when added alone. Further examination showed that the time-dependent PKA phosphorylation of GRK2 caused solely by rolipram was ablated by the PKA-selective inhibitor H89. Interestingly, despite the fact that 40% of total cAMP PDE activity in these cells is contributed by PDE3, addition of the
PDE3-specific inhibitor cilostamide failed to promote PKA phosphorylation of GRK, showing that this effect of GRK2 is regulated by PDE4. The involvement of PDE4 was confirmed using siRNA (small interfering RNA) that selectively ablated PDE4B and PDE4D subfamilies. Knockdown of either the PDE4B or the PDE4D isoform enhanced the ability of isoprenaline to cause PKA phosphorylation of GRK2, but was not sufficient to trigger this in unstimulated cells. However, siRNA-mediated knockdown of both PDE4D and PDE4B expression together did achieve such an increase in resting cells, thereby mimicking the effect of selective chemical inhibition with rolipram. In all cases, PKA phosphorylation of GRK2 was attenuated by H89.

**PDE4 accelerates membrane translocation of GRK2, subsequent phosphorylation of the β2Ar by GRK2 and recruitment of β-arrestin**

Isoprenaline challenge of HEK-293β2 cells resulted in the time-dependent membrane translocation of GRK2, which was accelerated and became more transient in the added presence of rolipram. Again, it was evident that rolipram pre-treatment sufficed to stimulate GRK2 translocation, which occurred in a time-dependent manner and was abolished by H89. Chemical inhibition of PDE4 also accelerated the phosphorylation of β2Ar by GRK. Once again, rolipram treatment alone caused time-dependent GRK2 phosphorylation of the β2Ar, corresponding with the observed recruitment of GRK2. This effect could also be elicited by concomitant siRNA knockdown of PDE4B and PDE4D together. As expected, isoprenaline triggered the transient membrane recruitment of β-arrestin and associated PDE4D isoforms (PDE4D5 ≫ PDE4D3 ≠ PDE4B2). This transient effect occurred more rapidly with rolipram pre-treatment and, indeed, we noted that rolipram alone sufficed to cause translocation of β-arrestin and PDE4D to membrane fractions.

**Rolipram triggers PKA phosphorylation of GRK2 in cardiac myocytes**

Isoprenaline caused the PKA phosphorylation of GRK2 and the membrane recruitment of GRK2 in cardiac myocytes. These responses were amplified by treatment with rolipram but could be triggered, albeit to a much lesser extent, by challenge with rolipram alone. These actions of rolipram were ablated by H89.

**Conclusions**

We have shown that the inactivation of PDE4, by using either a selective inhibitor or siRNA knockout, results in accelerated isoprenaline-induced GRK2 recruitment, GRK2 phosphorylation of the β2Ar and β-arrestin translocation to the β2Ar. Surprisingly, however, we have also shown that these effects can occur, albeit to a reduced extent, merely upon PDE4 inactivation and without any need for an agonist. That such effects can occur in the absence of agonist clearly challenges current dogma that states that the membrane recruitment of GRK2 is agonist-dependent.

It is now well established that GPCRs exist in a state of equilibrium between active and inactive states and that ligand binding drives this equilibrium towards the active state [8]. We envisage that a small basally active population of receptors exists which allows for the generation of a small pool of activated Gαs, which is responsible for basal adenylate cyclase activity, together with providing free Gβγ that is key to underpinning the membrane recruitment of GRK2. In HEK-293β2 cells, it would appear that the basal adenylate cyclase activity, with concomitant PDE4 inhibition, is sufficient to activate PKA that is able to act on GRK2. As PKA phosphorylation of GRK2 is known to increase its affinity for Gβγ, this modification will inevitably facilitate GRK2 recruitment to the membrane.

We conclude that PDE4 is pivotal in regulating the susceptibility of GRK2 to phosphorylation by PKA. Under agonist-stimulated conditions, PDE4 activity will influence the rate of agonist-triggered GRK2 recruitment by defining susceptibility to PKA phosphorylation. PDE4 therefore modulates β2Ar desensitization at the level of GRK2 phosphorylation, involving PDE4B and PDE4D pools, and at the level of phosphorylation of the β2Ar itself by AKAP79-tethered PKA, which is specifically regulated by β-arrestin-sequestered PDE4D5. However, in resting cells, we propose that PDE4 activity protects GRK2 from inappropriate phosphorylation by PKA that might have occurred through fluctuations in basal cAMP levels. Presumably, PDE4 activity sets a threshold for PKA phosphorylation of GRK2, which normally requires agonist challenge of the β2AR to breach. Thus PDE4 gates the action of PKA to phosphorylate GRK2. These observations, especially in cardiac myocytes, may have relevance in heart failure, as Pde4d gene inactivation in mice can lead to accelerated heart failure after myocardial infarction [9], and it is known that inhibition of GRK2 can be protective against cardiac dysfunction [10].

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**References**


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