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Review

Regulation of G protein-coupled receptor kinase subtypes by calcium sensor proteins

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Abstract

G protein-coupled receptor homologous desensitization is intrinsically related to the function of a class of S/T kinases named G protein-coupled receptor kinases (GRK). The GRK family is composed of six cloned members, named GRK1 to 6. Studies from different laboratories have demonstrated that different calcium sensor proteins (CSP) can selectively regulate the activity of GRK subtypes. In the presence of calcium, rhodopsin kinase (GRK1) is inhibited by the photoreceptor-specific CSP recoverin through direct binding. Several other recoverin homologues (including NCS 1, VILIP 1 and hippocalcin) are also able to inhibit GRK1. The ubiquitous calcium-binding protein calmodulin (CaM) can inhibit GRK5 with a high affinity (IC_{50} = 40–50 nM). A direct interaction between GRK5 and Ca^{2+}/CaM was documented and this binding does not influence the catalytic activity of the kinase, but rather reduced GRK5 binding to the membrane. These studies suggest that CSP act as functional analogues in mediating the regulation of different GRK subtypes by Ca^{2+}. This mechanism is, however, highly selective with respect to the GRK subtypes: while GRK1, but not GRK2 and GRK5, is regulated by recoverin and other NCS, GRK4, 5 and 6, that belong to the GRK4 subfamily, are potently inhibited by CaM, which had little or no effect on members of other GRK subfamilies. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: G protein coupled receptor; G protein coupled receptor kinase; Calcium sensor protein; Calmodulin

1. Introduction

The continuous communication among cells is fundamental to maintain the coherent function and specialization of the system they are a part of. Central for the maintenance of this homeostasis are cell surface receptors that receive and transduce inside the cells signals coming from outside. G protein-coupled receptors (GPCR) form the largest known family of cell surface receptors and share the characteristic structure of seven transmembrane domains. These receptors bind to a variety of agonists such as photons, neurotransmitters, hormones and chemokines thus regulating very diverse functions as vision, olfaction, neurotransmission, proliferation and immune processes. The signal transduction process triggered by agonist binding to GPCR consists of discrete steps and one of the earliest is the coupling of this complex to heterotrimeric G protein that dissociates in Gα and Gβγ subunits [1]. They activate or

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inhibit a number of effector enzymes like adenylyl cyclase, phospholipase C, ion channels or as recently identified c-Src and mitogen activated protein kinases (MAPK), resulting in a variety of cellular functions.

GPCR-mediated signal transduction is strictly regulated by multiple mechanisms acting at different levels of signal propagation. After agonist stimulation, the receptor is desensitized by G protein-coupled receptor kinase (GRK) phosphorylation and subsequent binding of arrestin proteins (homologous desensitization) [2–5]. The activated α subunit of the G protein (Gα) can in turn be inhibited by RGS (regulators of G protein signalling) proteins [6–8]. These RGS proteins are functionally homologous to the well known GAPs (GTPase activating proteins) acting on small G proteins such as Ras. In fact, RGS proteins interact with Gα, thus increasing its intrinsic GTPase activity.

Most of the studies on GPCR regulation by GRK have used GRK2 as a prototype kinase. It was shown by several laboratories that a variety of GPCR can be regulated by GRK2 likely through phosphorylation, although a phosphorylation-independent mechanism has been reported for desensitization of parathyroid hormone receptor [9,10].

The existence of multiple mechanisms that regulate GPCR signalling may have relevant functional consequences. For example, GRK which acts at the receptor level, should desensitize all the signalling pathways activated by one receptor, while the RGS proteins only desensitize the signalling activated by a given Gα protein. GRK2, besides desensitizing the receptor by phosphorylation, is able to selectively regulate Gαq. The GRK2 N-terminal domain directly interacts with the activated Gαq and regulates its signalling in a phosphorylation-independent manner [10]. GRK2 can regulate different GPCR-mediated responses by multiple mechanisms that depend on the coupling to different G proteins.

2. GPCR homologous desensitization

The mechanism of signal transduction must be highly regulated and one of the earlier regulatory events is homologous desensitization [2]. This is an adaptive mechanism of the receptors that favours the regaining of responsiveness after repeated stimuli over time. In fact, GPCR-mediated signal transduction must be properly regulated in order to prevent overstimulation, to achieve signal termination and to render the receptor responsive to subsequent stimuli [3]. The term 'homologous' means that the desensitization of the receptor is initiated by the activation of the same receptor by the agonist.

When the agonist binds to the receptor, it acquires the suitable conformation to be phosphorylated by one GRK. Phosphorylation can occur at the C-terminus of the receptor, as for rhodopsin and β_2-adrenoceptors, or at the third intracellular loop, as has been demonstrated for M_2-muscarinic receptor and α_2-adrenoceptor [11]. GRK-phosphorylated receptors are only minimally desensitized but this phosphorylation increases the affinity for arrestin. Binding of arrestin to the receptor then induces maximal homologous desensitization. The next event is the receptor sequestration in endosomal vesicles, where it will be degraded (downregulation) or dephosphorylated by specific phosphatases and then recycled to the membrane.

The biochemical details regarding the receptor regeneration are still unclear and actively investigated. In the case of rhodopsin, it has been demonstrated that the removal of the agonist from the receptor induces the dissociation of arrestin and allows the dephosphorylation by protein phosphatase 2A. For other GPCR it has been shown that the lower pH in the endosomal compartment is probably responsible for phosphatase activation and receptor conformational change that promotes arrestin disassembling [4].

Distinct from homologous desensitization is the process of heterologous desensitization which is mainly determined by second messenger-dependent protein kinase A (PKA) and protein kinase C (PKC). The main difference between the two processes is that activated PKA or PKC phosphorylates not only agonist-bound receptors, but also other different receptors [3].

3. The GRK gene family

The GRK family is composed of six cloned members named GRK1 to GRK6, according to the order of their identification (Fig. 1). GRK1, GRK2 and

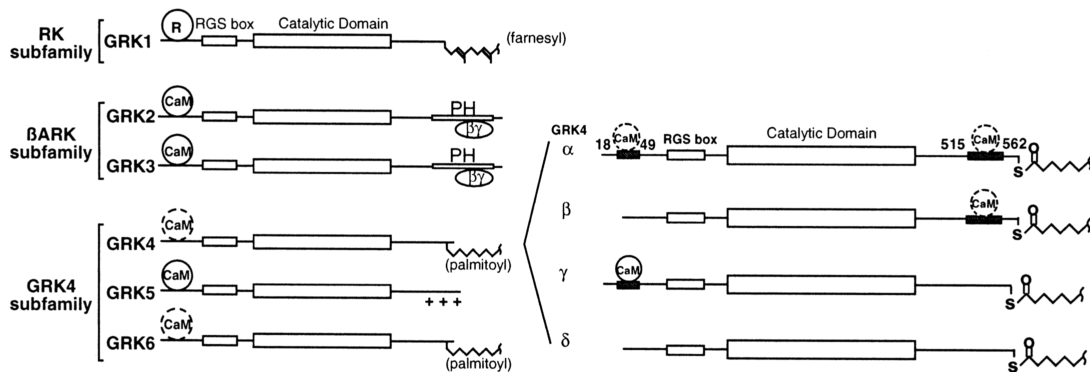

Fig. 1. GRK gene family. Schematic representation of the six GRK variants showing the common central catalytic domain (open box), the PH domain in GRK2 and GRK3, the CaM interacting region (CaM), and the putative 'RGS box'. Dotted CaM in GRK4 and GRK6 means that this domain is deduced by homology with GRK5. The principal mechanisms of membrane anchoring are indicated: farnesylation for GRK1, Gβγ binding for GRK2 and GRK3, palmitoylation for GRK4 and 6, and charges for GRK5 (+++).

GRK3 were previously named rhodopsin kinase, βARK1 and βARK2, respectively [3–5]. Importantly, GRK2, 3, 5 and 6 are widely distributed while GRK1 and GRK4 have selected sites of expression, in that GRK1 is localized exclusively in the retina, while GRK4 has been found so far in sperms only [12].

Based on the sequence homology the six GRK are classified in three subfamilies: GRK1 is alone in the first (RK subfamily), GRK2 and 3 form the second (βARK subfamily) while GRK4, 5 and 6 constitute the third (GRK4 subfamily) (Fig. 1). From an evolutionary point of view, RK and GRK4 subfamilies are more strictly related [3].

GRK2 consists of a central catalytic domain (F191-G443) a C-terminal domain (G443-L689) involved in membrane targeting and an N-terminal domain (M1-F191) whose functional role is poorly defined. GRK2 interacts directly with the dissociated Gβγ and this interaction is important for membrane translocation and activation of the kinase. The PH domain that is present within the GRK2 C-terminus is important for the binding to Gβγ. An RGS homology domain has been identified within the N-terminus of GRK2 [10] (Fig. 2).

The overall structure of GRK consists of a central core, which is the catalytic domain, flanked by the amino-terminal region and the carboxy-terminal region (Fig. 2). The catalytic domain that also contains the ATP-binding site is the most conserved region among all GRK subtypes. Sites of alternative splicing have been identified for some GRK subtypes and this topic is still a matter of investigation. No splice variants have been identified in human GRK2 and 3 and only one was found in GRK1. For human GRK4 two sites of alternative splicing have been identified, one at the N-terminal domain (exon 2) and the other at the C-terminal domain (exon 15), thus resulting in four splice variants (Fig. 1) [12,13]. Alternative splicing could be predicted for GRK5 and 6, as they are closely related to GRK4. So far, splice variants in rat GRK6, which are different from those of GRK4, have been demonstrated [14].

The membrane targeting domains of GRK are mainly located at the C-terminus, but the anchoring mechanisms are somewhat different. GRK1 have a farnesylation site, while a palmitoylation site is present in both GRK4 and GRK6 [4]. GRK2 and 3 have an extended C-terminus in which a PH domain is located and binding to Gβγ targets them to

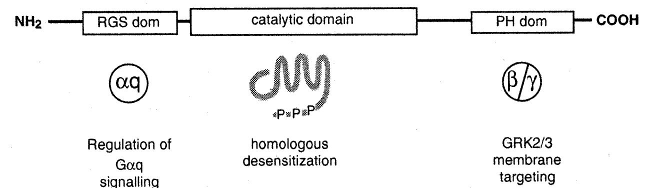

Fig. 2. Schematic representation of the three main functional domains of GRK2/3. The figure shows that the N-terminal domain, which contains an RGS homology region, interacts with Gαq. The catalytic central domain interacts and phosphorylates the GPCR. The N-terminus, which contains a PH domain, interacts with Gβγ.

the membrane (Fig. 1). The Gβγ-binding domain partially overlaps with the PH domain [15]. GRK5 likely binds to membrane phospholipids by positively charged amino acid clusters located at the C-terminus; on the other hand little is known so far about the N-terminal domain function.

4. The arrestin gene family

The arrestin family is composed of four members: one isoform is localized in the retina, another is in the cones, while β-arrestin1 and β-arrestin2 are ubiquitous [16]. Subtypes 1A and 1B of β-arrestin1 are distinguished by the inclusion or exclusion of a 24 base/8 amino acid cassette (exon 13) at the C-terminal region [17]. β-Arrestin2 also has two splice variants for the alternative splicing of 33 bases/11 amino acids in a region closer to the C-terminus [18]. Even arrestin, which is a single product including exon 13, was found either with or without exon 13 when expressed in peripheral blood leucocytes [17]. A new splice variant from human retina lacking exon 12 has been cloned.

In the classical view of the homologous desensitization, β-arrestin was considered as the GRK cofactor, which rendered the desensitization process complete. Now evidence is accumulating which indicates that β-arrestin has the important function to promote receptor sequestration. Depending on the type of GPCR, internalization can follow the clathrin coated or the clathrin uncoated vesicle pathways [19]. Thus the role of GRK phosphorylation of GPCR in the sequestration process is to favour β-arrestin binding. The β-arrestin1 activity in GPCR sequestration is regulated by phosphorylation. β-Arrestin1 is present in the cytosol as a phosphorylated form on its Ser-412 located at the C-terminus [20]. In the case of β_2-adrenoceptor, it has been demonstrated that, when β-arrestin1 is recruited to the plasma membrane by agonist stimulation, it is dephosphorylated. It has been shown that this dephosphorylation is important for the receptor sequestration, but it is not essential for receptor binding and desensitization.

Recent data have documented that the activation of MAP kinases by GPCR may require the endocytosis of the receptor [21,22]. The requirement of internalization for the activation of a transduction pathway is in striking contrast with the classical mechanisms, known for example for adenylyl cyclase or phospholipase C activation. From this novel point of view, GRK phosphorylation of agonist-bound GPCR and the binding of β-arrestin are not only the events of receptor desensitization, but they are permissive for MAP kinase activation.

5. Homologous desensitization is a regulated event

Homologous desensitization is a general mechanism regulating GPCR signal transduction. Given the very large number of GPCR (>1000) and the relatively low number of GRK (six genes), it appears evident that the interaction between these proteins is not based on a one-kinase-for-one-receptor rule. Most cell types have a variety of GPCR on their surface and they express more than one GRK that could phosphorylate and regulate these receptors. Additionally, different GPCR can be phosphorylated by the same GRK subtype, indicating that receptor specificity is not the key determinant for kinase-receptor interaction. Therefore it is conceivable to hypothesize the existence of intracellular mechanisms able to regulate the activity of GRK and to provide some degree of selectivity in the interaction between GPCR and receptor kinases.

Several mechanisms able to modulate the activity of GRK have been identified. As an initial step, the stimulated receptor (i.e. agonist-occupied) is able to activate GRK [23,24] by one domain which is exposed upon agonist binding. Two other potent regulators of GRK activity are PIP_2 and Gβγ, which are also important for the membrane targeting of these kinases [25–27]. PIP_2, which interacts directly with GRK and facilitates the membrane association of the kinase, enhances the ability of all the GRK subtypes to phosphorylate receptor substrate. In contrast the ability of Gβγ to enhance the kinase activity was exclusively seen with the βARK subfamily members GRK2 and GRK3. Only GRK2 and GRK3 have an extended C-terminus which contains the Gβγ binding site [28]. All the other GRKs have a shorter C-terminus lacking either the PH domain or the Gβγ binding sequence.

Intracellular calcium can modulate GRK activity

by different mechanisms. For example a raise of cytosolic calcium activates PKC which can phosphorylate different GRKs. Phosphorylation by PKC has opposite functional effects on GRK subtypes: GRK2 kinase activity is increased while GRK5 is substantially inhibited. Increased intracellular calcium also binds to and activates intracellular proteins known as calcium sensor proteins (CSP).

6. The calcium sensor proteins

Interaction of Ca^{2+} with a large number of Ca^{2+}-binding proteins represents one of the mechanism by which this second messenger controls many biological processes [29,30]. Calcium binds with high affinity to these Ca^{2+}-binding proteins and induces conformational changes that enable these proteins to interact and regulate a variety of targets. One class of these proteins shares a common Ca^{2+}-binding motif, the EF hand. This structural motif, first identified in the crystal structure of parvalbumin, consists of two perpendicularly placed α-helices (helices E and F in parvalbumin) and one interhelical loop, which together form a single Ca^{2+}-binding site. The mechanism of this molecular switch lies in the conformational change induced by Ca^{2+} binding. EF hand proteins with regulatory roles are often termed CSP, whereas those involved in Ca^{2+} buffering and transport are termed Ca^{2+} buffer proteins. CSP represent a heterogeneous class of proteins which includes calmodulin (CaM), neurone-specific calcium sensor proteins named neuronal calcium sensors (NCS), such as recoverin, visin-like protein (VILIP), neurocalcin, hippocalcin and the recently identified S100 family members. A new subfamily of retinal calcium-binding proteins (named CABP) has been recently identified [31].

7. Interactions between recoverin and GRK1

Recoverin is a 23 kDa myristoylated CSP (also called S-modulin in the frog) expressed predominantly in vertebrate photoreceptor cells. Kawamura [32] reported that S-modulin inhibits the phosphorylation of photoexcited rhodopsin in frog retina in a calcium-dependent manner. This resulted in a prolonged lifetime of catalytically active rhodopsin and therefore in a larger number of transducin molecules activated by rhodopsin. It was suggested that this effect could play a major role in light adaptation [33,34].

The molecular mechanism of recoverin inhibition of rhodopsin phosphorylation was subsequently elucidated [35,36]. It was found that recoverin acts directly on rhodopsin kinase (RK) to decrease its catalytic activity and that inhibition of rhodopsin phosphorylation by recoverin was Ca^{2+}-dependent. The covalently attached myristoyl residue enhanced the inhibitory effect of recoverin as determined in an assay of phosphorylation of urea-stripped rod outer segment (ROS) membranes by purified recombinant RK. The IC_{50} for myristoylated recoverin was 0.8 μM and the IC_{50} for nonacylated recoverin was 8 μM at saturating Ca^{2+} concentrations. Recoverin binds directly with high affinity to RK in a Ca^{2+}-dependent manner and this interaction is important for RK inhibition.

8. Interactions between CaM and the βARK subfamily

Following the evidence that GRK1 is regulated by recoverin, other studies were performed to test whether this is a general phenomenon with respect to other CSP and GRK subtypes. Initial studies were performed on CaM, which is an acidic protein that is considered the primary 'decoder' of Ca^{2+} information in the cell [37,38]. Three different laboratories found that GRK2 and GRK3 are inhibited by CaM in a calcium-dependent manner [39–42]. The IC_{50} was 2 μM. Since Gβγ is a binding target of Ca^{2+}/CaM and the binding of GRK2 and 3 to Gβγ is critical for the activation of these two GRK subtypes, it was proposed that Gβγ could represent the site of indirect interaction between GRK2 and 3 with Ca^{2+}/CaM. However, CaM was able to inhibit GRK2 kinase activity even in the absence of Gβγ, indicating that the effect of CaM is not due to sequestration of Gβγ from GRK2. The mechanism of this inhibition likely involves the direct interaction between CaM and GRK2. Two distinct CaM-binding sites located within the N- and C-terminal regions of GRK2 [43] were identified by performing

a surface plasmon resonance (SPR) analysis of GRK2-CaM interaction. The two sites are located within residues 1–88 and 593–689 of GRK2.

9. Interactions between CaM and the GRK4 subfamily

Further studies demonstrated that among all the GRK subtypes CaM interacts more potently with GRK5 [39–42]. The IC_{50} was 40–50 nM, indicating that CaM is approx. 50-fold more potent in inhibiting GRK5 kinase activity as compared to GRK2 and 3. The other two members of this GRK subfamily, GRK4 and GRK6, were also strongly inhibited by CaM. The inhibitory effect of CaM on GRK4 [12] and GRK6 [41] was approx. 3-fold lower than that on GRK5 and approx. 30-fold more potent than that on GRK2. For GRK4 the calculated IC_{50} was 80 nM.

The high affinity of CaM for GRK5 suggests that this interaction can be physiologically relevant. This effect was then extensively characterized [39–44]. A direct interaction between GRK5 and Ca^{2+}/CaM was demonstrated using CaM-conjugated Sepharose 4B and confirmed using SPR technology on a BIAcore instrument. The two CaM-binding sites on GRK5 are located within residues 20–39 in the N-terminus and 540–578 in the C-terminus [43]. CaM binding does not influence the catalytic activity of GRK5 as demonstrated by the lack of an inhibitory effect on its phosphorylating activity on the soluble GRK substrate casein. Instead, Ca^{2+}/CaM significantly reduced kinase binding to the membrane and to phospholipid vesicles. It was suggested that CaM can directly compete for both the phospholipid- and receptor-binding sites of GRK5. It was also demonstrated that CaM activated GRK5 autophosphorylation.

The following model of GRK5 regulation by CaM was proposed. At resting Ca^{2+} concentrations GRK5 is active and able to phosphorylate agonist-occupied receptors. When a cell is stimulated and intracellular Ca^{2+} levels rise, CaM binds to GRK5 and inhibits directly receptor phosphorylation. However, since CaM-stimulated autophosphorylation also inhibits GRK5 activity, the kinase should remain inhibited even when Ca^{2+} levels go down and CaM dissociates from the enzyme. Presumably, GRK5 will eventually be dephosphorylated and return to its basal level of activity. Thus, CaM-stimulated autophosphorylation may prolong the inhibitory effect of a transient increase of intracellular Ca^{2+} levels on GRK5. A similar regulatory cycle has been demonstrated for CaM-kinase II [45].

10. Selectivity in the regulation of GRK by CSP

These studies suggest that CSP act as functional analogues in mediating the regulation of different GRK subtypes by Ca^{2+}. Preliminary studies also indicate that S100 protein is able to inhibit GRK2 in a calcium-dependent manner [46], suggesting that several classes of EF hand CSP can regulate GRK activity. This mechanism is, however, highly selective with respect to the different CSP and GRK subtypes (see Table 1).

Recoverin, which inhibited GRK1 phosphorylating activity by direct binding to this kinase, in parallel experiments did not interact with GRK2 and was ineffective on ROS phosphorylation by this kinase subtype. Several other recoverin analogues, including NCS 1, VILIP 1 and hippocalcin, are also able to inhibit GRK1 but they do not inhibit GRK5 kinase activity. An analysis of VILIP in olfactory neurones indicated that in these cells VILIP does not interfere with GRK2. Taken together these data indicate that recoverin and other NCS can selectively inhibit

Table 1
Selective inhibition of GRK subtypes by CSP

	Recoverin	VILIP	NCS-1	CaM	CABP[a]	References
GRK1	++	++	++	−	nd	[35,47]
GRK2	−	−	−	+	−	[39,40,42][b]
GRK3	nd	–	nd	+	nd	[39,49]
GRK4	nd	nd	nd	+++	nd	[12]
GRK5	−	−	−	++++	++	[35,48,42]
GRK6	nd	nd	nd	+++	nd	[41]

CSP were ineffective (−) or able to inhibit the indicated GRK subtypes with different degrees of potency (+). nd, not determined.

[a]CABP is the name of a recently identified family of CBP that includes five members with different inhibitory activities on GRK5.

[b]M. Sallese, L. Iacovelli and A. De Blasi, unpublished data.

GRK1 and do not affect the other GRK subtypes. By contrast CaM strongly inhibits GRK4 subfamily members while it has little (for GRK2 and 3) or no (for GRK1) effect on the other GRKs. In addition, in a recent study the group of Palczewski identified five new calcium-binding proteins, named CABP, that inhibit GRK5-mediated phosphorylation of rhodopsin with different affinities but did not affect GRK2 activity (Table 1) [31].

11. Functional implications

The physiological role of recoverin-GRK1 interaction was intensively investigated. Calcium is a fundamental mediator of the phototransduction process, it activates several Ca^{2+}-binding proteins, which in turn modulate the activity of important enzymes in the phototransduction cascades [50,51]. Rhodopsin activation by light decreases the retinal concentration of Ca^{2+}, thus activating several transduction pathways all leading to the to negative regulation of the effect of light. These feedback mechanisms are important to accelerate the recovery of the response to light and diminish the effect of illumination, thus enabling the cell to adapt to background light. The reduction of Ca^{2+} levels following rhodopsin activation by light will inactivate recoverin that releases GRK1, thus allowing the inhibitory effect of rhodopsin phosphorylation to proceed. GRK1 activation reduces the lifetime of rhodopsin in the active conformation, which is one mechanism of light adaptation. This functional model could be an oversimplification of the physiology of vision since there are few reports that do not fit with the proposed model. Knockout mice for the recoverin gene show no major effects in their response to photon absorption. However, the removal of the recoverin gene could result in an unpredictable compensation by other components of the phototransduction cascade. Since recoverin inhibits the phosphorylation of dark-adapted rhodopsin better than that of bleached rhodopsin it was proposed that recoverin-dependent regulation of GRK1 plays a role in preventing the enzyme from unwanted phosphorylation of dark-adapted rhodopsin (this phenomenon is known as 'high-gain' phosphorylation) [52,53]. GRK2, GRK3 and GRK5 regulate the vast majority of GPCRs and some of those,

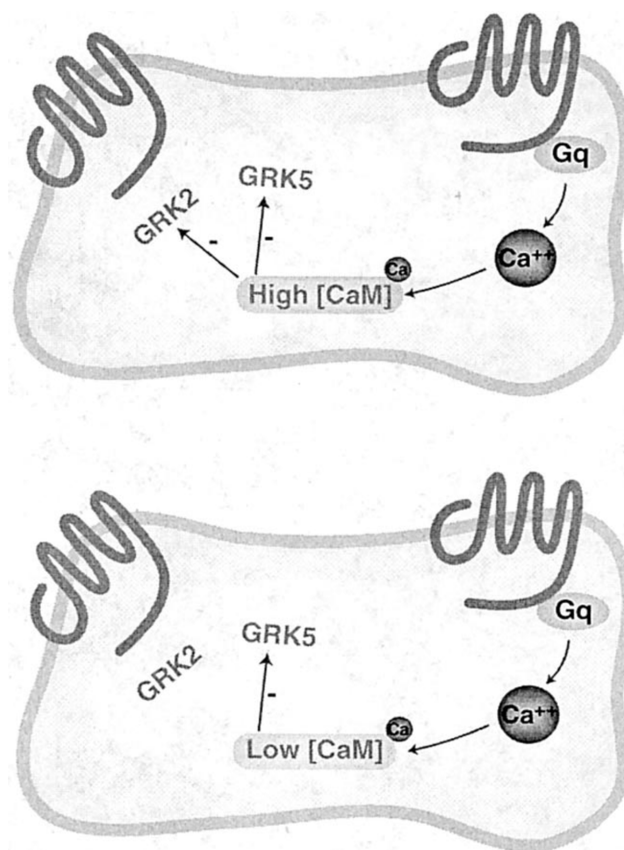

Fig. 3. Selective regulation of GRK5 versus GRK2 by CaM. Activation of the phospholipase C/IP_3 pathway increases the intracellular Ca^{2+} level. Ca^{2+} activated CaM could potentially inhibit GRK2- and GRK5-dependent receptor phosphorylation. Likely in tissues where CaM levels are high GRK2 and GRK5 activity is affected, while in tissues where CaM levels are low only GRK5 activity can be regulated.

such as substance P, angiotensin II, and 5-HT_2 receptors are coupled to fluctuations in intracellular Ca^{2+}. For this class of GPCRs CaM can provide a feedback mechanism to modify homologous desensitization. Moreover, different GRK could potentially regulate the same receptor when it is occupied by the agonist. CSP could work addressing which kinase will desensitize the activated receptor, e.g. activation of a GPCR coupled to Ca^{2+} signalling can regulate the phosphorylating activity of these GRK subtypes, thus modulating the efficiency of a different receptor regulating in a heterologous manner the homologous desensitization (Fig. 3). To support this view only few functional data are available: in transfected CHO cells the M_2-muscarinic receptor internalization is reduced by pretreatment with Ca^{2+} ionophore

A23187 [40]. M_2-Muscarinic receptor sequestration is considered specific of and sensitive to the GRK2 activity [54], hence these results indicate that GRK2 activity in intact cells is suppressed by increased Ca^{2+} concentrations.

12. Concluding remarks

A novel mechanism for crosstalk in signal transduction is revealed by the identification of intracellular pathways that can modify the GRK/arrestin homologous desensitization machinery. The mechanisms so far identified include PKC and Ca^{2+}-binding proteins, involved in the regulation of GRKs and cAMP-PKA involved in the regulation of arrestins [55]. The existence of other pathways cannot be excluded. Since PKC, PKA and Ca^{2+}-binding proteins can be activated by a large variety of receptor agonists, it can be envisaged that the functional state of the GRK/arrestin machinery is under continuous regulation in response to the fluctuating extracellular environment, thus providing the cell with another homeostatic mechanism. A challenging point is to define how these different effectors work in integrated systems. The role of intracellular calcium in the modulation of regulatory proteins is becoming more and more relevant. For example, other CSPs could be involved in the regulation of GPCR-mediated signalling. Additionally a new class of phosphatases named G protein-coupled receptor phosphatases (GRP) has recently been identified. These GRP dephosphorylate desensitized receptors, thus favouring resensitization [56–58]. Interestingly these GRPs possess two EF hands and their activity is calcium dependent.

All these observations support the idea of a complex integrated network, working towards efficient signalling. The identification of the players and the definition of their relative function represent a major goal in this field.

The existence and the functional importance of these regulatory pathways may not be universal, but it could depend on factors such as receptor selectivity and cell type specificity. For example GRK/arrestin regulation of different receptors, and the regulation of the same receptor in different cell types may have different levels of significance [59].

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