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Mechanism of isoproterenol-induced RGS2 up-regulation in astrocytes

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Abstract

Regulators of G protein signaling (RGSs) are inducibly expressed in response to various stimuli and the up-regulation of RGSs leads to significant decreases in GPCR responsiveness. Isoproterenol, an adrenergic receptor agonist, stimulated RGS2 mRNA in C6 rat astrocytoma cells. The up-regulation of RGS2 mRNA was abrogated by genistein, a protein tyrosine kinase inhibitor (PTK), and by broad-spectrum protein kinase C (PKC) inhibitors (staurosporine and GF109203X). α-Adrenergic antagonist (prazocin), β-adrenergic antagonist (prazocin), and pertussis toxin only partially blocked the RGS2 up-regulation, suggesting that the RGS2 up-regulation is concomitantly mediated by $G\alpha_i$, $G\alpha_s$, and $G\alpha_q$. It is interesting to note that SB203580, a potent p38 mitogen-activated protein kinase (MAPK) inhibitor, completely inhibited the isoproterenol-mediated RGS2 expression. In addition, isoproterenol also markedly stimulated RGS2 mRNA in rat primary astrocytes, which were sensitive to SB203580 and staurosporine. Therefore, our data suggest that adrenergic receptor-mediated signaling (induced by isoproterenol) may be involved in the regulation of RGS2 expression in astrocytes via activating PTK, PKC, and p38 MAPK.

Keywords: RGS2; Astrocytes; Isoproterenol; Adrenergic receptor; Protein kinase C; P38 mitogen-activated protein kinase

The transduction of signals across cellular membranes is a biological phenomenon of extreme importance that involves the control of cellular proliferation and differentiation [1,2]. Classically, these pathways consist of three components: seven transmembrane receptors, heterotrimeric G proteins, and various types of effectors, including enzymes that generate second messengers, ion channels, and protein kinase cascades [3]. A fourth component of the G protein signaling system has recently become appreciated; the regulator of G protein signaling (RGS) family of proteins [4,5]. RGS is the negative regulator of G protein signaling. Efforts to identify a new RGS have extended this family to approximately 30 subtypes of RGS proteins. Biochemical studies indicate that all RGS proteins have potential in attenuating G protein signaling by accelerating the rate of the GTP hydrolysis through G protein α-subunits called GTPase-accelerating protein (GAP) [6,7]. RGS2 is expressed at a low level but this can be increased by ligands of GPCR, including angiotensin II and parathyroid hormone presumably to provide a feedback mechanism to down-regulate receptor-mediated signaling [8–10]. Several stressor transiently increased RGS2 mRNA similarly to immediately early genes like c-fos [11–13]. On the other hand, astrocytes are the major cell constituents of the central nervous system (CNS) and occupy 20–30% of brain volume. Astrocytes, which are named for their stellate appearance, project foot processes that envelop the basement membrane of neurons, synapses, and capillaries,

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thereby consisting of a blood–brain barrier (BBB) [14]. Astrocytes play a role in the metabolism of neurotransmitters (e.g., serotonin) and in mechanisms of neurotransmitter uptake that protect neurons from injury during glutamate toxicity [15]. Astrocytes act as a mediator of inflammatory responses and are implicated in the pathophysiology of neurodegenerative diseases including Alzheimer disease (AD) and multiple sclerosis (MS) [16]. It is known that cultured astrocytes express a wide variety of G protein coupled receptors, including β2-adrenoceptor, α1-adrenoceptor, and chemokine receptors [17,18]. The β2-adrenoceptors have been known to be expressed more conspicuously in the reactive astrocytes at the boundary of cerebral infarction [18]. The reactive astrocytes lead to an increase in the levels of intracellular cyclic adenosin-5-monophosphate (cAMP) [17]. It is interesting to note that astrocytes in MS patients lack the β2-adrenoceptor with the mechanism unknown. Moreover, the activation of the β2-adrenoceptor by isoproterenol leads to the morphological differentiation of primary astrocytes [19], an increased production of the nerve growth factor by astrocytoma cells [20], and the reactive astrocytes become sensitive to the cytotoxicity of 7β-hydroxysterol [21]. These phenomena seemed to be due to the increased level of intracellular cAMP, which thereby activates the astrocytes.

Therefore, it is known that the expression of RGS genes is regulated by Ca^{2+}/calmodulin, PKA, and PKC via either GPCR signaling or the direct regulation of protein kinase. Based upon the above, we evaluated whether isoproterenol regulated the expression of RGS2 and its up-regulation mechanism in astrocytes by using C6 astrocytoma cells and primary astrocytes.

Materials and methods

Materials. Rat C6 astrocytoma cells were obtained from the Korean Cell Line Bank (Seoul, Korea). Isoproterenol, prazocin, propranolol, PD98059, SB202190, and SP600125 were from Tocris (Bristol, UK). PTX, genstein, and staurosporine were purchased from Sigma (St. Louis, MO).

Cell culture and transfection. (on Supplementary Materials)

Extraction of total RNA. Total RNA from culture cells was prepared by adding easy-BLUE total RNA extraction reagent (iNtRON Co., Seoul), according to the manufacturer's protocol. The total RNA was solubilized with 0.1% diethylpyrocarbonate (DEPC)-treated DDW and was stored at −70 °C until used.

Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) amplification. (on Supplementary Materials)

Results

The expression of RGS2 in rat astrocytoma C6 cells

Since G-protein signaling plays an important role in the pathophysiology of the CNS, we intended to determine whether isoproterenol, acting via GPCR, modulated the expression of RGS transcripts in transformed C6 cell and primary astrocytes. First, we assessed whether RGS2 mRNA is expressed in C6 astrocytoma cells. The transcript of RGS2 mRNA was detectable in RT-PCR by using the poly dT_{18} primer and gene-specific PCR primers. We found that isoproterenol elevated RGS2 mRNA levels in a time- and dose-dependent manner (Figs. 1A and B). As shown in Fig. 1A, isoproterenol enhanced the expression of RGS2 mRNA in as little as 30 min and within 60 min at the most. The expression recovered in the incubation time that followed the basal levels of RGS2 mRNA. In addition, as shown in Fig. 1B, isoproterenol stimulated the expression of RGS2 mRNA in a dose-dependent manner (0.01 to

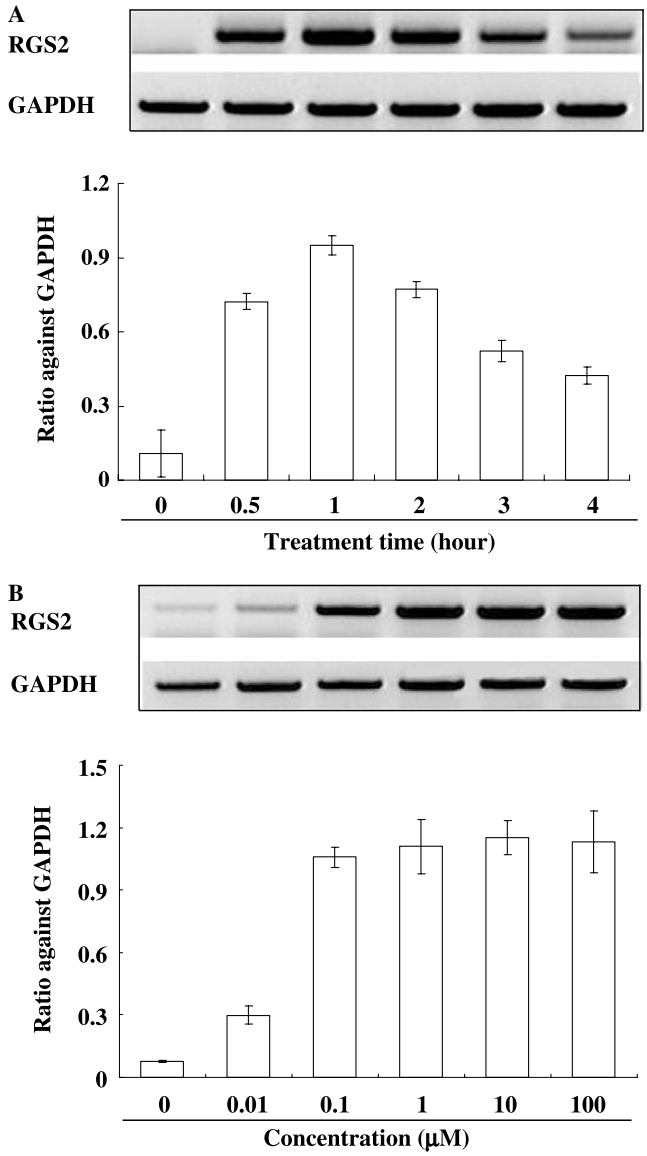

Fig. 1. Isoproterenol induced the elevation of RGS2 transcripts in a time- (A) and dose-dependent manner (B) in C6 astrocytoma cells. (A,B) The cells were stimulated with the indicated concentration levels of isoproterenol (ISP, 1 μM) and were incubated for various times or for 1 h. The preparation of total RNA and RT-PCR was described in Materials and methods. The PCR products were run in a 1% agarose gel using an electrophoresis kit (Bio-Rad Co.), and then the products were visualized and the expression ratio against GAPDH was calculated using the Eagle Eye system (Promega).

100 μM). The expression level of RGS2 mRNA reached the plateau status at 1 μM. We, therefore, carried out the following experiments using 1 μM of isoproterenol. Actinomycin D was employed to evaluate the contribution of new transcriptional control in these regulatory mechanisms. Actinomycin D (5 μM) completely reduced the expression of RGS2 which was induced by 1 μM of isoproterenol (Supplementary Fig. 1).

The involvement of the G protein complex, but not single subtype of the G protein, was found in the isoproterenol-regulated RGS2 expression

Since astrocytes express α- and β-adrenoceptors [22,23] and isoproterenol could couple to both subtypes of adrenoceptors, to a different extent of affinity, we intended to approach the blocking of the expression of RGS2 mRNA with the broad-spectrum antagonists of α- and β-adrenoceptors. As shown in Fig. 2A, prazocin (1 μM), an antagonist of α-adrenoceptor, which is broadly coupled to the $G\alpha_q$ protein, markedly inhibited the up-regulation of RGS2 mRNA, and propranolol (1 μM), an antagonist of β-adrenoceptor, which is coupled to the $G\alpha_s$ protein, significantly inhibited the expression of RGS2 transcripts induced by 1 μM of isoproterenol. This suggests that both α-adrenoceptor- $G\alpha_q$ and β-adrenoceptor- $G\alpha_s$ signaling pathways play an important role in the isoproterenol-induced RGS2 up-regulation.

Pretreatment of the cells with PTX partially prevented the up-regulation of RGS2 mRNA in the isoproterenol-treated C6 astrocytoma cells for 1 h (Fig. 2B). PTX is known to interfere with the dissociation of $G_{\beta\gamma}$ subunits from the heterotrimeric G protein complex via the ADP ribosylation of the $G_{\alpha i/o}$ subunits and is a good tool which clarifies the involvement of $G\alpha_{i/o}$ subunits in GPCR signaling. This finding, therefore, demonstrates that the RGS2 regulation by isoproterenol is dependent in part on the activation of PTX-sensitive $G\alpha_{i/o}$ proteins.

The effects of PKA and PKC inhibitors

Next, we determined whether a wide variety of protein kinase inhibitors affected the regulation of RGS2 mRNA. In order to begin to define the downstream signaling mechanism for the isoproterenol-induced increase in the expression of RGS2 mRNA, we focused on the role of PKA, the major effector kinase that mediates the action of cAMP. Surprisingly, two different pharmacological inhibitors of PKA, H89 (10 μM) and H-7 (100 μM), showed only a limited inhibition (<20%) of the isoproterenol-induced increase in the expression of RGS2 mRNA (Supplementary Fig. 2). These data suggested that isoproterenol-induced RGS2 expression is predominantly regulated by PKA-independent pathways in C6 astrocytoma cells. Subsequent studies were designed to examine this possibility. We intend to determine whether PKC takes a role in the signaling of

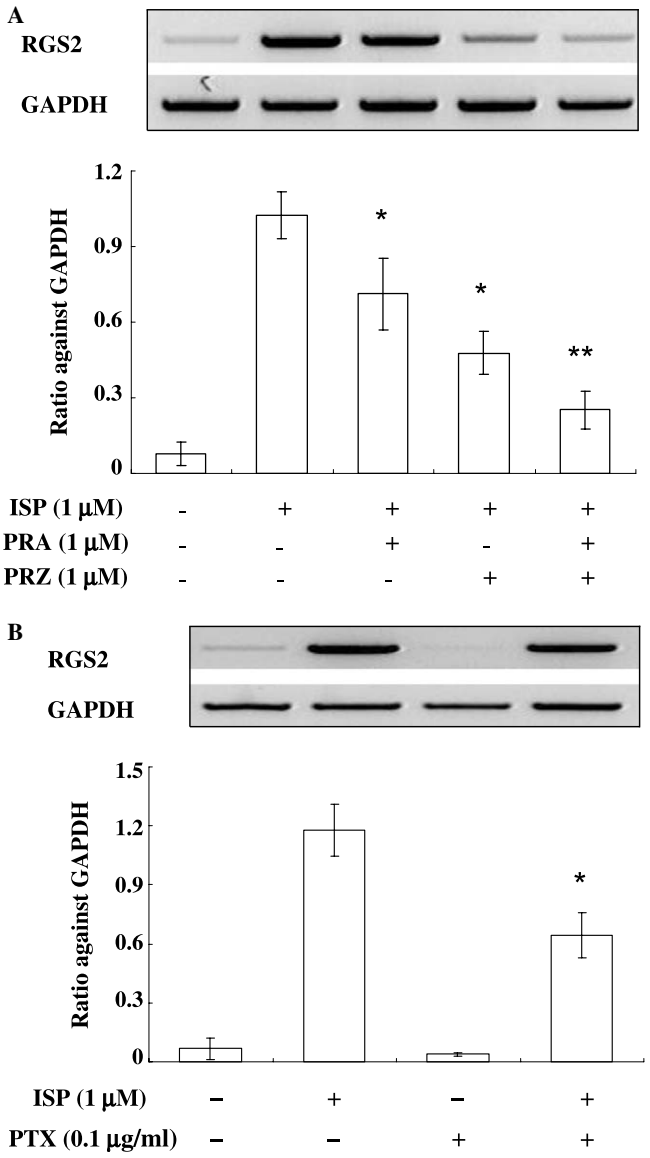

Fig. 2. The effects of adrenoceptor blockers, prazocin and propranolol, and PTX on the isoproterenol-induced RGS2 expression in C6 astrocytoma cells. The up-regulation of RGS2 mRNA by isoproterenol is partially dependent on the $G\alpha_s$, $G\alpha_{i/o}$, and $G\alpha_q$. (A) The pretreatment of prazocin (PRZ, 1 μM) or propranolol (PRA, 1 μM) for 30 min and consecutive addition of isoproterenol (ISP, 1 μM) are described in Fig. 1. (B) The C6 cells were added with PTX (0.1 μg/ml) and were incubated for 18 h. The preparation of the total RNA and RT-PCR was described in Materials and methods. The calculation of the RGS2 expression against GAPDH is provided in Fig. 1. *$P < 0.05$ versus control, **$P < 0.01$ versus control.

isoproterenol-induced RGS2 regulation. As shown in Fig. 3, general PKC inhibitors, such as staurosporine and GF109203X, significantly suppressed RGS2 up-regulation. In addition, staurosporine suppressed isoproterenol-induced RGS2 up-regulation in a dose-dependent manner (Supplementary Fig. 3). These studies have suggested that under these conditions, PKC played a more important role in RGS2 regulation than PKA.

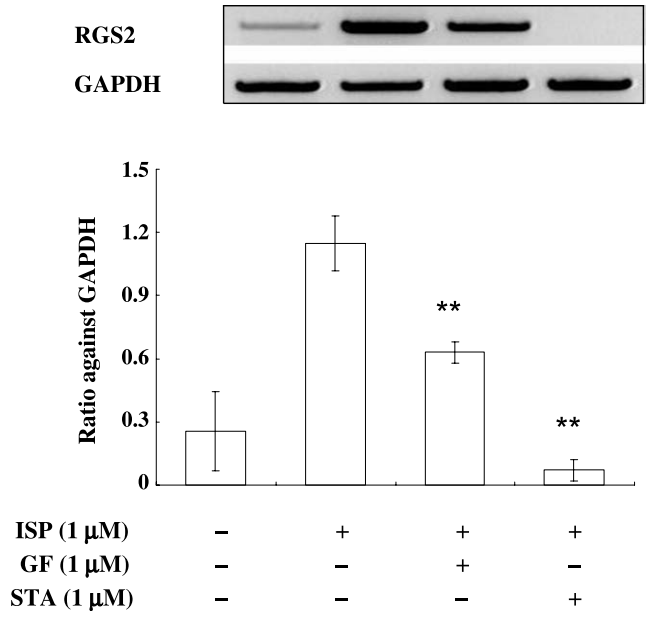

Fig. 3. The effects of PKC inhibitors, GF109203X, and staurosporine on the isoproterenol-induced RGS2 expression in C6 astrocytoma cells. The pretreatment of GF109203X (GF, 1 μM) and staurosporine (STA, 1 μM) and the addition of isoproterenol (1 μM) were performed for Fig. 1. The preparations for total RNA and RT-PCR were described in Materials and methods. The calculation of the RGS2 expression, against GAPDH, is described in Fig. 1. $^{**}P < 0.01$ versus control.

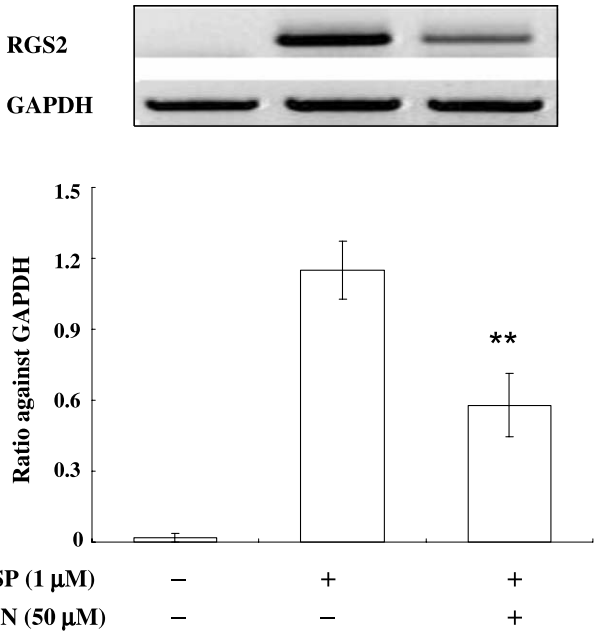

Fig. 4. The effects of a non-receptor tyrosine kinase inhibitor, genistein, on the isoproterenol-induced RGS2 up-regulation in C6 astrocytoma cells. The pretreatment of genistein (GEN, 50 μM) and the addition of isoproterenol (ISP, 1 μM) are shown in Fig. 1. The preparation of the total RNA and RT-PCR are described in Materials and methods. The calculation of the RGS2 expression against GAPDH is provided in Fig. 1. $^{**}P < 0.01$ versus control.

The effect of the non-receptor protein kinase inhibitor genistein on RGS2 regulation by isoproterenol

Fig. 4 shows that genistein (50 μM) significantly reversed the up-regulation of RGS2 transcripts induced by isoproterenol, suggesting that the non-receptor tyrosine kinase is involved in the regulation of the RGS2 transcript. In order to elucidate the involvement of Src kinase, one of the representative non-receptor tyrosine kinases, we obtained the dominant negative mutant of Src kinase (i.e., kinase deficient c-Src) [24] and determined whether the deletion of this kinase affected RGS2 gene expression, by isoproterenol, using a co-transfection technique. Transfection of the dominant negative mutant of Src kinase, however, did not block isoproterenol effect on RGS2 expression, indicating that src kinase is not involved in this event (data not shown).

Involvement of p38 MAPK in the isoproterenol-induced RGS2 regulation

The MAPK family (ERK 1/2, JNK, and p38 MAPK) has an important role in the downstream component of extracellular signaling. Among them, the p38 MAPK inhibitor, SB203580, almost completely suppressed the isoproterenol-induced RGS2 up-regulation, but the ERK inhibitor PD98059 and the JNK inhibitor SP600125 did not (Fig. 5). The above data suggest that p38 MAPK is involved in the regulation of RGS2 mRNA induced by iso-

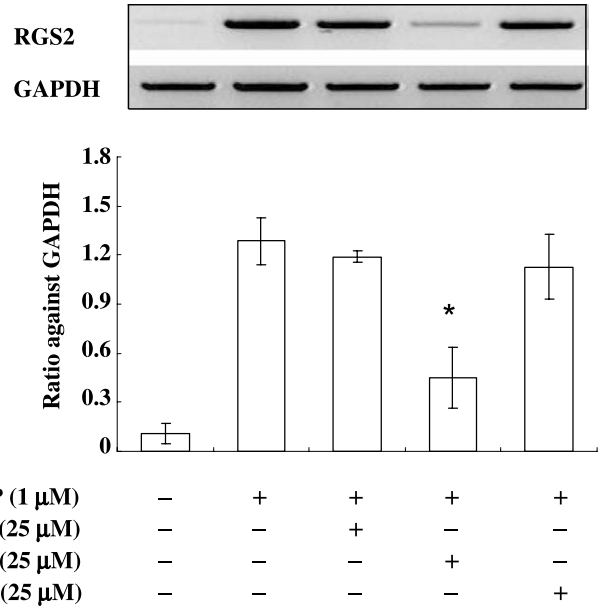

Fig. 5. The effects of MAPK inhibitors, SP10, SB203580, and PD98059 on the regulation of RGS2 transcripts in C6 astrocytoma cells. The pretreatment of inhibitors SP60125 (SP, 25 μM), SB203580 (SB, 25 μM), and PD98059 (PD, 25 μM), and the addition of isoproterenol (ISP, 1 μM) are shown in Fig. 1. The preparation of total RNA and RT-PCR was described in Materials and methods. The calculation of the RGS2 expression against GAPDH is described in Fig. 1. $^{*}P < 0.05$ versus control.

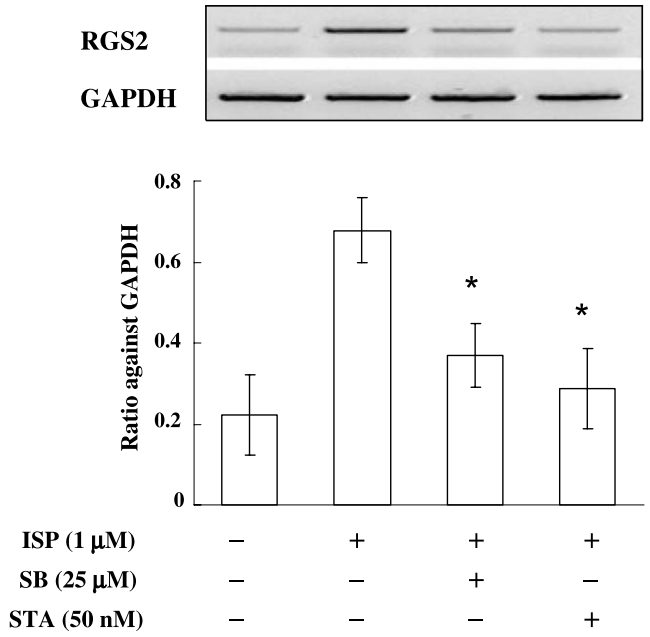

Fig. 6. Isoproterenol induced the elevation of RGS2 transcripts in primary astrocytes, which is sensitive to p38 MAPK inhibitor, SB203580, and PKC inhibitor, staurosporine. The pretreatment of SB203580 (SB, 25 μM) and staurosporine (STA, 50 nM), and the addition of isoproterenol (1 μM) are shown in Fig. 1. The preparation of the total RNA and RT-PCR was described in Materials and methods. The calculation of RGS2's expression against GAPDH is described in Fig. 1. *$P < 0.05$ versus control.

proterenol and that p38 MAPK could be an important candidate that is relayed from extracellular activation (with isoproterenol) to intranuclear RGS2 regulation.

The up-regulation of RGS2 mRNA induced by isoproterenol in the rat primary astrocytes

To confirm whether isoproterenol effect in C6 astroma cells is shown in primary astrocytes, the same experiments were conducted with rat primary astrocytes. As shown in Fig. 6, isoproterenol increased the expression of the RGS2 transcript three times more than that of the basal level. This up-regulation was markedly abrogated by the p38 MAPK inhibitor, SB203580, which was in agreement with C6 astrocytoma cells. In addition, a general PKC inhibitor, staurosporine (0.05 μM), completely reversed the isoproterenol-induced RGS2 regulation into the basal level. These data suggested that like C6 astrocytoma, primary astrocytes are also isoproterenol-responsive.

Discussion

There are three major findings in this study. First, we have demonstrated that isoproterenol, acting through both α- and β-adrenoceptors, enhanced the expression of RGS2 mRNA in both transformed C6 astrocytoma cells and rat primary astrocytes. Second and most importantly, we have shown that the stimulatory effect of isoproterenol on the expression of RGS2 transcripts is mediated by a p38 MAPK pathway, rather than by the ERK or JNK pathways. Finally, the present study indicated that the PKC pathway may play a major role in the isoproterenol-induced RGS2 regulation more than the PKA pathway, especially in the early stages of RGS2 induction (i.e., between 30 and 60 min of treatment).

In the astrocytes, the classic pattern of isoproterenol-induced signaling is known to increase cAMP production, leading to the activation of PKA [25–27]. Moreover, some laboratories reported that the phosphorylation of β-adrenoceptor by cAMP-PKA switched its predominant coupling from a stimulatory guanine nucleotide regulatory protein (G_s) to the inhibitory guanine nucleotide regulatory protein (G_i) [28,29]. Through the use of selective kinase inhibitors, however, our results clearly show that both G_q and G_i signaling components seem to be required for the subsequent transcriptional activation of the expression of RGS2 mRNA, which was induced by 1 μM of isoproterenol. We hypothesized that the early activation of the PKC-p38 MAPK pathway leads to the up-regulation of RGS2 mRNA, and then the PKA pathway would play an important role in the regulation of RGS2 transcripts in the late phases of isoproterenol signaling in astrocytes.

The regulation of the expression of RGS2 mRNA with isoproterenol in transformed astrocytoma cells and primary astrocytes

We have first chosen the C6 rat astrocytoma cells in this experiment due to the relatively simple transfection and expression of endogenous α- and β-adrenoceptors [25,26,30]. It is well known that astrocytes play an important physical and physiological role in the development of neurodegenerative disease including AD and MS. In addition, astrocytes are composed of a blood–brain barrier as the end-feet, which provides the brain with protection from toxins and harmful metabolites found in blood. Moreover, RGS is the negative regulator of G protein signaling, which is closely related to the pathophysiology of GPCR-related diseases in the CNS. Therefore it is worthwhile to determine the regulation of RGS by isoproterenol (via the activation of GPCR) in astrocytes. As shown in Fig. 1, isoproterenol (1 μM) enhanced the expression of RGS2 mRNA in a time-dependent manner, which reached maximal activity at 1 h and diminished afterwards. The kinetic control of RGS2 mRNA, induced by isoproterenol, is somewhat different from that induced by forskolin (FSK, 10 μM). FSK, a direct activator of all known subtypes of adenylyl cyclases (AC, Type I to IX), enhanced the expression of RGS2 mRNA with maximal activity at 2 h and stimulation was retained up to 4 h (Kim and Rhee, in preparation). The rapidity with which the message is induced is one of the most striking characteristics of the isoproterenol-induction of RGS2 mRNA, similar to the angiotensin II-stimulation in rat vascular smooth muscle cells [8]. We have also shown that actinomycin D completely blocked

the RGS2 up-regulation, suggesting that this is transcriptional-dependent (Supplementary Fig. 1).

Involvement of various G_α -protein complexes in the isoproterenol-induced RGS2 expression

It is well known that isoproterenol is a potent, non-selective β-adrenergic agonist with a low affinity for α-adrenoceptors. In addition to that β_2-adrenoceptors are coupled to $G\alpha_s$ protein, the activated form of the receptor is associated with the $G\alpha_i$ protein [31,32]. Therefore, to clarify the signaling pathway from receptor to G-protein subunits, several inhibitors related to this pathway were employed. As Figs. 2A and B and Supplementary Fig. 2 show, the inhibitors of $G\alpha_s$- (i.e., propranolol), $G\alpha q$- (i.e., prazocin), and $G\alpha_i$-pathway (i.e., PTX) partially blocked isoproterenol-induced RGS2 mRNA regulation. Although the selectivity of these inhibitors toward each target is deficient, it suggests that isoproterenol-mediated response seems to require various G proteins, rather than a specific G protein, linked to boosting the level of second messengers. It is also reported that G protein βγ dimmer (dissociated from the $G\alpha_s$ protein) mediates the inhibitory effect of isoproterenol on the generation of NADPH-dependent H_2O_2 in human adipocyte plasma membranes [33]. Transfection of βark, G protein βγ scavenger, however, did not affect the isoproterenol-induced RGS2 up-regulation in our system, indicating that G protein βγ subunits, which are dissociated from possibly $G\alpha_i$, $G\alpha_s$, or $G\alpha_q$, might not be involved in the modulation of RGS2 mRNA. The interference of PTX, a $G_{\beta\gamma}$ subunit dissociation inhibitor, on the up-regulation of RGS2 mRNA by isoproterenol (Fig. 2B) appears to indicate that PTX-sensitive $G\alpha_{i/o}$ proteins may participate in the isoproterenol-mediated RGS2 regulation.

Several points that (1) the activated PKA induces the phosphorylation of β-adrenoceptor and the switching from $G\alpha_s$ to $G\alpha_i$ coupling [1,34], resulting in the activation of the tyrosine kinase-Ras-Raf1-ERK1/2 pathway by βγ-subunits released from $G\alpha_i$ [34,35], and (2) activated Gs-mediated PKA activation would stimulate p38 MAPK [36] led us to exploring downstream signaling including PKA, PKC, PTK, and MAPK.

The effect of inhibitors of PKA and PKC

It has been considered that the second messengers such as cAMP, diacylglycerol, and inositol trisphosphate would mediate the isoproterenol-induced up-regulation of RGS2 mRNA. Two different pharmacological inhibitors of PKA, H89 (10 μM), and H-7 (100 μM), showed only limited inhibition (<25%) of the isoproterenol-induced increase in the expression of RGS2 mRNA. These data suggested that the isoproterenol-induced RGS2 expression is predominantly regulated by PKA-independent pathways in C6 astrocytoma cells. Subsequent studies were designed to examine whether PKC plays a role in the signaling of the isoproterenol-induced RGS2 regulation. Staurosporine, a potent and broad-spectrum inhibitor of protein kinase, strongly blocked the RGS2 up-regulation (Fig. 3 and Supplementary Fig. 3). GF109203X, another highly selective and general protein kinase C inhibitor, significantly suppressed the isoproterenol-induced RGS2 regulation (Fig. 3A). Rottlerin (10 μM), however, a novel PKC (e.g., PKC_δ) inhibitor, did not affect the RGS2 regulation (data not shown). These studies suggested that the PKC pathway (especially the classic subtype of PKC) may play an important role in adrenergic receptor-mediated response.

The effects of the inhibitors of mitogen-activated protein kinase (MAPK)

Isoproterenol effect on RGS2 transcripts was completely inhibited by 25 μM SB203580 but not SP600125 and PD98059 (Fig. 5). These findings show that the specific activation of the p38 MAPK pathway may be resulted in the stimulation of RGS2 mRNA in C6 astrocytoma cells and in primary astrocytes. The activation of adrenergic receptors, with isoproterenol, in cultured astrocytes is thought to reproduce the morphological and functional changes, similarly shown in the case of cell stress [37,38]. It was worth to note that oxidative stress, with hydrogen peroxide and heat shock, also increased the RGS2 mRNA levels [12]. Moreover, the activation of p38 MAPK in astrocytes is also reported under the oxidative stress conditions [39–41]. Therefore, previous findings and our results suggest that RGS2 may be induced under stress conditions via a p38 MAPK pathway.

The effects of the non-receptor protein kinase inhibitor genistein on the RGS2 expression of isoproterenol

Transmembrane signaling traffic through GPCR has been reported to be grown by classic G protein effectors, such as AC and phospholipase C, as well as novel proteins, such as receptor and non-receptor tyrosine kinase for the activation of MAPK cascades [1,28]. Therefore, we examined whether protein tyrosine kinase pathways are involved in isoproterenol-induced RGS2 regulation. Fig. 4 shows that PTK may be one of major signaling enzymes in this event. Thus, genistein, a broad-spectrum tyrosine kinase inhibitor, strongly suppressed isoproterenol-mediated RGS up-regulation. Several experiments, therefore, were added to verify the importance of PTK. For this purpose, the involvement of receptor tyrosine kinse (e.g., epidermal growth factor receptor kinase) and non-receptor tyrosine kinase (e.g., Src) was examined using AG490, a specific EGFR inhibitor, and the src dominant-negative mutant as well as constitutively active src mutants. However, these types of PTK were not involved in the isoproterenol-mediated RGS2 expression (data not shown).

In summary, the idea that up-regulation of the RGS leads to significant decreases in GPCR responsiveness brought us to assess whether isoproterenol regulated the

expression of RGS2 in astrocytes, by using C6 astrocytoma cells and primary astrocyte culturing. Isoproterenol stimulated the expression of RGS2 in a dose-and time-dependent manner. The up-regulation of the RGS2 gene, however, was abrogated by genestein, a protein tyrosine kinase inhibitor, and by staurosporine and GF109203X, potent and broad-spectrum PKC inhibitors. Either treatment of prazocin, an α-adrenergic antagonist or propranolol, a β-adrenergic antagonist, partially blocked the isoproterenol-induced RGS2 expression. Overnight treatment with PTX partially affected the RGS2 expression, suggesting that the isoproterenol-induced RGS2 expression is concomitantly mediated by $G\alpha_i$-, $G\alpha_s$-, and $G\alpha_q$-subtype, and not by a specific G-protein subtype in itself. It is interesting to note that SB203580, a potent p38 mitogen-activated protein kinase (MAPK) inhibitor, completely inhibited RGS2 mRNA which was stimulated by isoproterenol, but not by PD98059 and SP600125, extracellular regulated kinase 1/2 (ERK1/2) and Janus-like kinase (JNK) inhibitors, respectively. Therefore, our data suggest that adrenergic receptor (isoproterenol)-mediated dynamic regulation of RGS2 mRNA may play an important role in the development of GPCR-related and astrocyte-mediated brain disorders such as AD and MS.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.08.061.

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