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Adenylyl cyclase type-VIII activity is regulated by $G_{\beta\gamma}$ subunits

Debora Steiner[a], Daniella Saya[a], Ester Schallmach[a], William F. Simonds[b], Zvi Vogel[a,*]

[a]*Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel*
[b]*Metabolic Diseases Branch, NIDDK, National Institutes of Health, Bethesda, MD, 20892, United States*

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Abstract

The Ca^{2+}-activated adenylyl cyclase type VIII (AC-VIII) has been implicated in several forms of neural plasticity, including drug addiction and learning and memory. It has not been clear whether $G_{i/o}$ proteins and G-protein coupled receptors regulate the activity of AC-VIII. Here we show in intact mammalian cell system that AC-VIII is inhibited by μ-opioid receptor activation and that this inhibition is pertussis toxin sensitive. Moreover, we show that $G_{\beta\gamma}$ subunits inhibit AC-VIII activity, while constitutively active $\alpha_{i/o}$ subunits do not. Different G_{β} isoforms varied in their efficacies, with $G_{\beta1\gamma2}$ or $G_{\beta2\gamma2}$ being more efficient than $G_{\beta3\gamma2}$ and $G_{\beta4\gamma2}$, while $G_{\beta5}$ (transfected with γ_2) had no effect. As for the G_{γ} subunits, $G_{\beta1}$ inhibited AC-VIII activity in the presence of all γ subunits tested except for γ_5 that had only a marginal activity. Moreover, cotransfection with proteins known to serve as scavengers of $G_{\beta\gamma}$ dimers, or to reduce $G_{\beta\gamma}$ plasma membrane anchorage, markedly attenuated the μ-opioid receptor-induced inhibition of AC-VIII. These results demonstrate that $G_{\beta\gamma}$ (originating from agonist activation of these receptors) and probably not $G_{\alpha i/o}$ subunits are involved in the agonist inhibition of AC-VIII.
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1. Introduction

Stimulation of seven-transmembrane domain inhibitory receptors (e.g., μ-, δ-, and κ-opioid, m_2-and m_4-muscarinic, D_2-dopaminergic) activates $G_{i/o}$ proteins, as a result of which the $G_{\alpha i/o}$ subunit exchanges its GDP for GTP and both $G_{\alpha i/o}$ subunits and $G_{\beta\gamma}$ dimers become available to interact with effectors [1–4]. For example, both $G_{\alpha i/o}$ subunits and $G_{\beta\gamma}$ dimers were shown to interact with various adenylyl cyclase (AC) isozymes, and $G_{\beta\gamma}$ subunits were reported to lead to either inhibition or stimulation of AC activity, depending on the AC isozyme in question [5–7].

Nine mammalian membranous AC isozymes have been cloned and shown to differ in their tissue distribution and biochemical properties, including sensitivity to $\alpha_{i/o}$, α_s, $G_{\beta\gamma}$ dimers, Ca^{2+}concentration, and PKC activation [5–9]. All of the nine AC isozymes are large proteins (1080–1248 amino acids) each comprising two cassettes of six transmembrane-spanning domains, with each cassette being followed by a large cytoplasmic domain [7,10]. The C-terminus part of these cytoplasmic domains, the C_{1b} and C_{2b} regions, are not conserved and are speculated to reflect the regulatory features of the specific AC isozymes [10–13].

AC-VIII was shown to be stimulated by Ca^{2+} ions [14]. The Ca^{2+}-stimulatory effect seems to be mediated via calmodulin loosely bound to specific domains of the AC molecule [15,16]. We showed that ACVIII activity could be synergistically stimulated by the Ca^{2+} ionophore ionomycin added together with forskolin (FS) [17]. Regarding the regulation of AC-VIII by $G_{i/o}$PCRs, Nielsen et al. [31] showed that in HEK293 cells, AC-VIII activity was not significantly affected by the activation of either somatostatin or D_2L-dopaminergic receptors. In contrast, our group showed that AC-VIII transfected into COS-7 cells is inhibited by activation of various inhibitory receptors, including the μ-opioid, CB_1 and CB_2 cannabinoid, and D_2L-dopaminergic receptors [17–19]. However, it was

* Corresponding author. Tel.: +972 8 934 2402; fax: +972 8 934 4131.
E-mail address: zvi.vogel@weizmann.ac.il (Z. Vogel).

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unclear whether $G_{\alpha i/o}$ subunits or $G_{\beta\gamma}$ dimers participate in this agonist-induced inhibition of AC-VIII activity. To determine the role of $G_{\alpha i/o}$, we transfected COS-7 cells with cDNAs encoding AC-VIII along with cDNAs encoding the various constitutively active $G_{\alpha i/o}$ isoforms. In addition, we studied the effect of various G_β and G_γ subunits as well as of $G_{\beta\gamma}$ scavengers on AC-VIII activity. We found that AC-VIII could be inhibited by various combinations of $G_{\beta\gamma}$ dimers, but not by the constitutively active $G_{\alpha i/o}$ subunits.

2. Materials and methods

2.1. Materials

[^{3}H-2]adenine (30.0 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Morphine was obtained from the National Institute of Drug Abuse, Research Technology Branch (Rockville, MD). Ionomycin and the phosphodiesterase inhibitors 1-methyl-3-isobutylxanthine (IBMX) and RO-20-1724, were from Calbiochem (La Jolla, CA). Forskolin (FS) was from Sigma (St. Louis, MO). Tissue culture reagents were from Gibco (Grand Island, NY). Pertussis toxin (PTX) was from List Biological Laboratories (Campbell, CA).

2.2. Plasmids

The rat μ-opioid receptor in pCMV-neo [20] was kindly provided by Dr. Huda Akil (University of Michigan, Ann Arbor). α-transducin ($G_{\alpha t}$), the human lymphocyte CD8 receptor and a chimera of the C′ terminus of β-adrenergic receptor kinase (βARK-C) and CD8 (CD8-βARK-C) in pCDNA1-Amp [21] were generously provided by Dr. S. Gutkind (NIDR, NIH, Bethesda, MD). AC-VIII, described by Cali et al. [14], in the mammalian expression vector pCMV5-neo was kindly provided by Prof. J. Krupinski (Bristol-Myers Squibb, Princeton, NJ). The various G_α (wild-type and constitutively active), G_β and G_γ cDNAs in pcDNA3.1+ were purchased from the Guthrie cDNA Resource Center (Sayre, PA). $G_{\gamma 2}$C68S (a mutant of $G_{\gamma 2}$ which cannot undergo prenylation) in pcDM8 was previously described [22,23].

2.3. Cell cultures and transfection methods

COS-7 cells, cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 5% fetal calf serum, were transiently transfected in 10-cm plates with plasmids containing appropriate cDNAs using the DEAE-dextran method, as previously described [17,24]. This method was optimized to reach high transfection efficiency (ranging from 40% to 80%). Twenty-four hours following transfection, the cells from each 10-cm plate were transferred to 24-well plates, and 24 h later, assayed for AC activity.

2.4. AC activity

Cells cultured in 24-well plates were preincubated for 2 h with 0.25 ml of 5 μCi/ml of ^{3}H adenine, and then washed three times with DMEM containing 20 mM HEPES (pH 7.4) and 0.1 mg/ml bovine serum albumin. Phosphodiesterase inhibitors (0.5 mM RO-20-1724 together with 0.5 mM IBMX) were then added, followed by the addition of reagents, which stimulate AC activity, such as ionomycin (1 μM) and FS (0.1 μM), in the presence or absence of morphine, a μ-opioid receptor agonist. After 10 min of incubation, the medium was removed and the reaction terminated with 2.5% perchloric acid. Following that, the supernatant was neutralized and applied to a two-step column separation procedure, as previously described [24].

2.5. Data analysis

Data are expressed as means ± S.E.M. of three independent experiments, each performed in triplicate. In each experiment, the data (in cpm) was transformed to percent of control activity and the percentages of the various experiments averaged. Results were analyzed using the Student's *t*-test.

3. Results

COS-7 cells were transfected with the cDNAs for the μ-opioid receptor and the AC type VIII. AC-VIII activity was then determined following activation with ionomycin, FS or a mixture of both (in the presence or absence of the opiate agonist morphine). As shown in Fig. 1a, ionomycin (1 μM) stimulated AC-VIII activity by 6.6-fold, FS (0.1 μM) by 5.5-fold and the mixture of ionomycin (1 μM) and FS (0.1 μM) by 19.2-fold (see also [17]). Morphine (1 μM) added simultaneously with ionomycin and FS inhibited AC-VIII activity by 50–60% via all the three activation procedures (Fig. 1a and b). Fig. 2 shows that the effect of morphine is dose dependent. Half-maximal effect was observed at 0.8 nM of morphine, a concentration that is in agreement with the Kd of morphine to the μ receptor [25]. In addition, the inhibitory effect of morphine was completely blocked by the opioid antagonist naloxone (data not shown).

To determine the involvement of $G_{i/o}$ proteins in AC-VIII regulation by morphine, we pretreated the cells with PTX and activated AC-VIII with a mixture of ionomycin+FS. PTX had a small inhibitory effect on the level of stimulation of AC-VIII by ionomycin+FS (see legend to Fig. 2). However, it completely abolished the inhibition of AC-VIII by the opiate treatment, demonstrating that the morphine-induced inhibition of AC-VIII is mediated via PTX-sensitive $G_{i/o}$ proteins. It is known that PTX, via the ADP ribosylation of $G_{\alpha i/o}$, neutralizes this subunit, but in addition prevents the dissociation of the $G_{\beta\gamma}$ dimer from the ADP-ribosylated heterotrimeric $G_{i/o}$ complex. We therefore

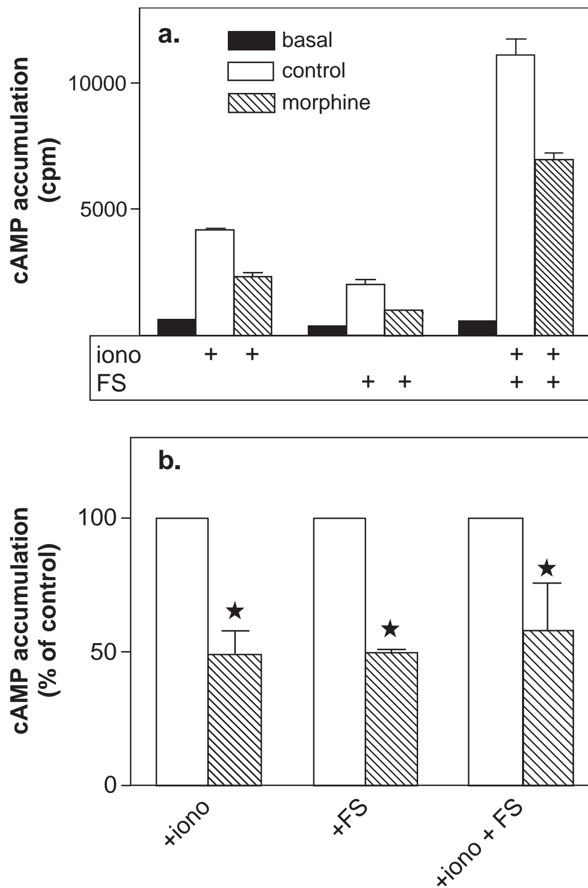

Fig. 1. Effect of opiates on AC-VIII stimulation by ionomycin and forskolin. COS-7 cells were transfected with 1 μg μ-opioid receptor and 2 μg AC-VIII cDNAs. cAMP accumulation was determined with or without stimulation of AC activity using 1 μM ionomycin (iono), 0.1 μM forskolin (FS), or a mixture of 1 μM iono+0.1 μM FS. (a) A representative experiment (values expressed in cpm of [^{3}H]cAMP formed) showing AC-VIII unstimulated activity (basal), stimulated AC-VIII activity (control) and following 10 min incubation with 1 μM morphine (morphine). (b) Shows the mean±S.E.M. of three experiments; 100% represents the amount of [^{3}H]cAMP formed in the absence of morphine. ★$p<0.01$ with respect to control.

decided to determine the roles of various $G_{\alpha i/o}$ subunits and $G_{\beta\gamma}$ dimers in the regulation of AC-VIII.

To determine if the agonist-induced inhibition of AC-VIII is mediated via $G_{\alpha i/o}$ proteins, we cotransfected AC-VIII together with various wt and constitutively active isoforms of these subunits, and AC-VIII activity was determined following activation with ionomycin+FS. Three $G_{\alpha i}$ (α_{i1}, α_{i2} and α_{i3}), two $G_{\alpha o}$ (α_{oA} and α_{oB}) and the PTX insensitive $G_{\alpha z}$ isoforms were tested. As a control, we perfomed the same experiment on cells transfected with AC-V and stimulated with FS. As shown in Fig. 3a, AC-VIII was not inhibited by any of the wildtype (i.e., non-activated) inhibitory G_α subunits. In contrast, the activity of AC-VIII was even stimulated by their presence. The same result was observed with cells transfected with AC-V (Fig. 3b). This result is in agreement with our previous finding that molecules that interfere with the activity of $G_{\beta\gamma}$ increase AC-V activity [24]. In addition, AC-VIII was not inhibited by any of the various constitutively active $G_{\alpha i/o}$ subunits tested. The effect of $G_{\alpha i1}$ Q204L, $G_{\alpha i2}$ Q205L and $G_{\alpha i3}$ Q204L was similar to that of the corresponding $G_{\alpha i}$ wt (Fig. 3a). A similar result was obtained with constitutively active $G_{\alpha oB}$ (Q205L), while the activity of AC-VIII in the presence of constitutively active $G_{\alpha oA}$ (Q205L) and $G_{\alpha z}$ (Q205L) was similar to that of control. The fact that neither of the constitutively active inhibitory α subunits showed inhibition of AC-VIII suggests that AC-VIII is not regulated by any of these $G_{\alpha i/o}$ subunits. The control experiment with AC-V (Fig. 3b) demonstrates that all the constitutively active α subunits were successfully expressed in the cells, and that all of them are actively inhibiting AC-V activity although with different efficacies (α_{i2} and α_{oB} being the most efficient).

To determine the possible role of $G_{\beta\gamma}$ dimers in the inhibition of AC-VIII, COS cells were cotransfected with AC-VIII cDNA, and different concentrations of $G_{\beta1}$ cDNA in the presence or absence of $G_{\gamma2}$ cDNA. Such a cotransfection increases the amount of $G_{\beta1\gamma2}$ dimers in the cell membrane [23]. As seen in Fig. 4, the mixture of $G_{\beta1}$ and $G_{\gamma2}$ ($G_{\beta1+\gamma2}$) inhibited the activity of AC-VIII, reaching a maximal inhibition of ~66% when the concentration of $G_{\beta1}$ cDNA was above 1 μg per 10-cm culture plate. A half-maximal effect was observed with ~250 ng of transfected $G_{\beta1}$ cDNA. The effect of $G_{\beta1}$ alone (without γ_2) reached a maximal inhibition of ~40%, demonstrating that the effect of $G_{\beta1+\gamma2}$ was much larger than that of $G_{\beta1}$ alone. On the other hand, $G_{\gamma2}$ alone did not have any effect on AC-VIII activity, a result which is in agreement with our previous report showing that transfection of γ_2 alone (without the β subunit) does not lead to stable γ_2 expression [23].

At the next step, we studied the effect of opioid receptor activation in the presence of cotransfected β_1 and γ_2. Both

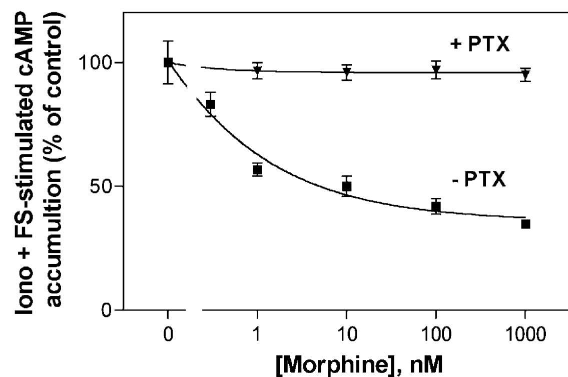

Fig. 2. Inhibition of AC-VIII by μ-receptor activation is blocked by PTX. COS-7 cells were cotransfected with the cDNAs for μ-opioid receptor (1 μg) and AC-VIII (2 μg). AC activity was stimulated with 1 μM iono+0.1 μM FS in the presence of the indicated concentrations of morphine. When indicated, the cells were preincubated for 20 h with 100 ng/ml PTX. The data represent the mean±S.E.M. of three experiments. 100% represents the amount of [^{3}H]cAMP formed in the absence of morphine (11794±1284 cpm for −PTX and 8850±1025 for +PTX, respectively).

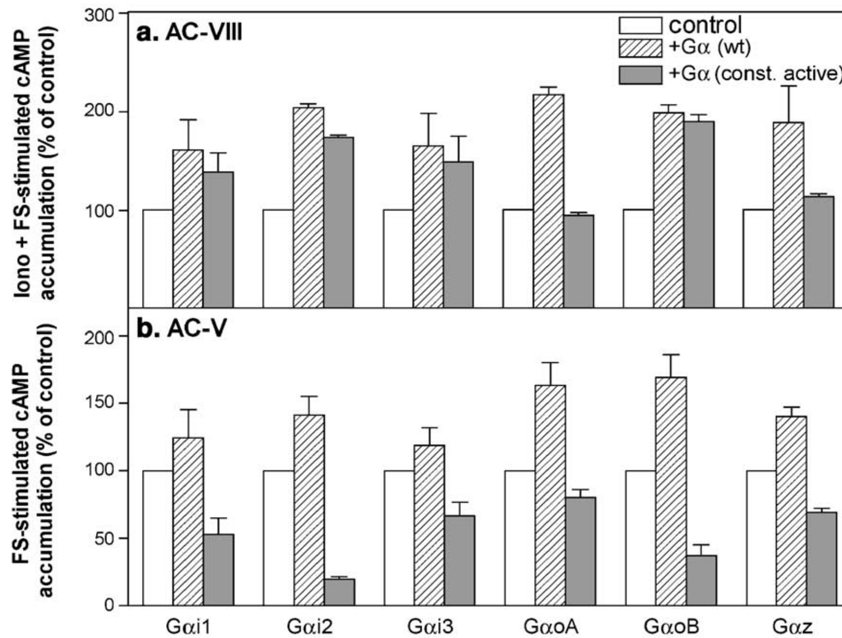

Fig. 3. Effects of wild-type and constitutively active $G_{\alpha i/o}$ isoforms on the activity of AC-VIII and of AC-V. Cells were transfected with 2 μg of the cDNAs for AC-VIII or AC-V, and with the cDNAs for either wt or constitutively active mutants of human $G_{\alpha i1}$, $G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha oA}$, $G_{\alpha oB}$ or $G_{\alpha z}$ (1 μg each). (a) AC-VIII activity was stimulated with 1 μM iono+0.1 μM FS. (b) AC-V activity was stimulated with 1 μM FS. 100% represents the amount of [^{3}H]cAMP formed in the absence of added $G_{\alpha i/o}$ subunits. Data are expressed as mean±S.E.M. of triplicate determinations.

$G_{\beta 1+\gamma 2}$ and opiate exposure led to AC-VIII inhibition (Fig. 5a). However, in the presence of $\beta_{1+\gamma 2}$, the activation of the opioid receptor did not lead to any further inhibition of AC-VIII. This can be better observed when the activity of AC-VIII in the absence of opiate treatment was normalized to 100% (Fig. 5b). This result is expected as the amount of $G_{\beta\gamma}$ dimers that are released upon receptor activation should be small compared with the amount of $G_{\beta 1\gamma 2}$ present in the cell following $\beta_{1+\gamma 2}$ transfection.

Five G_β and twelve G_γ isoforms have been cloned to date [2,26]. To study the effect of the various G_β subunits on AC-VIII activity, we cotransfected COS-7 cells with AC-VIII, $G_{\gamma 2}$ and each of the five G_β subunits. Fig. 6 shows that $G_{\beta 1+\gamma 2}$ and $G_{\beta 2+\gamma 2}$ were the most effective combinations to inhibit the ionomycin+FS-stimulated AC-VIII activity (inhibition of ~69% and 67%, respectively). The $G_{\beta 3+\gamma 2}$ and $G_{\beta 4+\gamma 2}$ combinations were less effective, showing 40% and 26% inhibition, respectively. On the other hand, $G_{\beta 5}$, when transfected with $G_{\gamma 2}$, was ineffective in inhibiting the activity of AC-VIII.

At the next step, we examined the effect of the different G_γ (in the presence of β_1) on the activity of AC-VIII (Fig. 7). We found that most G_γ subunits enhanced the inhibition of $G_{\beta 1}$. However, $G_{\gamma 5}$ was relatively inefficient and the inhibition of AC-VIII was not significantly affected by its presence.

As another approach to determine the role of $G_{\beta\gamma}$ in the inhibition of AC-VIII, we transfected the cells with plasmids which encode for molecules which either bind very tightly to free $G_{\beta\gamma}$ dimers or remove them from the plasma membrane and therefore interfere with the function of endogenous $G_{\beta\gamma}$ [27–29]. Three such $G_{\beta\gamma}$ scavengers were used in this experiment: (i) $G_{\alpha t}$ which binds $G_{\beta\gamma}$ but does not directly modulate AC activity; (ii) a chimera encoding the CD8 receptor (which allows anchoring to the membrane) and the carboxy terminus of βARK (which binds βγ) [30]; (iii) $G_{\gamma 2}$(C68S), a $G_{\gamma 2}$ mutant which lost its prenylation site and thus the capacity to anchor $G_{\beta\gamma}$ to the membrane. The use of this mutant will cause mislocation of the $G_{\beta\gamma}$ complex to the cytosol [23,28]. The results (Fig. 8) show that in cells transfected with the $G_{\beta\gamma}$ scavengers, the ability of μ receptor activation to inhibit ionomycin+FS-stimulated AC-VIII activity was markedly reduced. Similar results were also obtained when AC-VIII was activated by

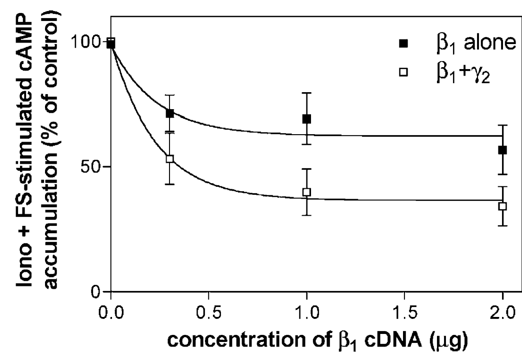

Fig. 4. Effect of $G_{\beta 1}$ and $G_{\gamma 2}$ on AC-VIII activity. COS-7 cells were transfected with 2 μg of the cDNA of AC-VIII, together with the indicated concentrations of $G_{\beta 1}$ cDNAs, in the presence or absence of 2 μg of $G_{\gamma 2}$ cDNA. Iono+FS-stimulated cAMP accumulation is expressed as percentage, where 100% represents control activity in the absence of $G_{\beta 1}$. Data are expressed as mean±S.E.M. of triplicate determinations of five experiments.

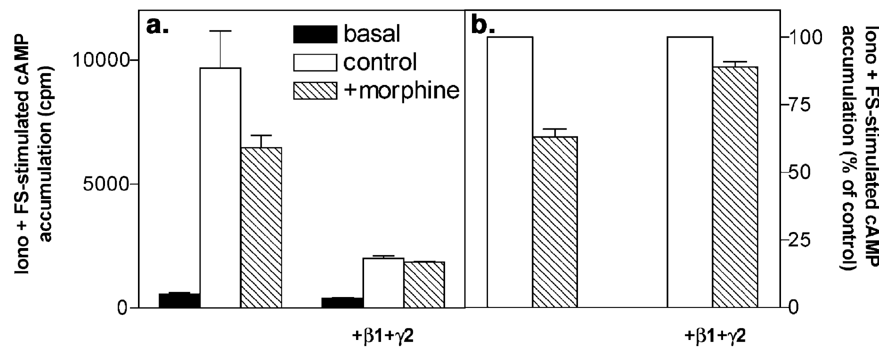

Fig. 5. Effect of $G_{\beta 1+\gamma 2}$ on the opiate-induced inhibition of AC-VIII. COS-7 cells were transfected with cDNAs as follows: 1 μg μ-receptor, 2 μg AC-VIII and where indicated 2 μg of β_1 and 2 μg of γ_2. (a) A representative experiment (values expressed in cpm of [^{3}H]cAMP formed) showing unstimulated AC-VIII activity (basal), ionomycin+FS-stimulated AC activity (control) and AC activity obtained in the presence of 1 μM morphine (morphine). (b) The figure shows the mean±S.E.M. of three experiments (performed as in (a)). The 100% value represents iono+FS-stimulated AC-VIII activity in the absence of morphine.

either FS or ionomycin alone (data not shown). Fig. 8b shows the percentage of loss of AC-VIII inhibition by the scavengers at the various morphine concentrations. It shows that the reversal of the inhibition by $G_{\beta\gamma}$ scavengers is more pronounced at low morphine concentrations. At 10 nM, morphine-induced inhibition was reduced by 49%, 52% and 44%, while at 1 μM morphine it was reduced by 35%, 32% and 22%, by $G_{\alpha t}$, $G_{\gamma 2}$C68S and CD8-βARK, respectively. Taken together, these results indicate that $G_{\beta\gamma}$ dimers inhibit the activity of AC-VIII and suggest that $G_{\beta\gamma}$ dimers, released upon receptor activation, have an important role in $G_{i/o}$PCR inhibition of AC-VIII.

4. Discussion

In this study, we show that agonist activation of μ-opioid receptor inhibits AC-VIII in a dose-dependent manner and that this inhibition is sensitive to PTX, revealing that this effect on AC-VIII activity is mediated via $G_{i/o}$ proteins. This result differs from that of Nielsen et al. [31] who reported that A23187-stimulated AC-VIII activity in HEK 293 cells was at most only very slightly inhibited by agonists of the inhibitory somastotatin and D_2L dopamine receptors, respectively. This difference in the capacity of AC-VIII to be inhibited by $G_{i/o}$PCR activation could be due to differences in membrane receptor density as well as in the different composition of $G_{i/o}$ protein subunits present in the different cell systems (see, for example, [32–34]).

It was previously shown that the inhibition by α subunits of $G_{i/o}$PCRs is AC isozyme specific. Activated $\alpha_{i/o}$ subunits were shown to inhibit the activity of AC-I, V and VI and had no effect on AC-II [35]. To study whether activated α-subunits could regulate AC-VIII, we transfected the cells with cDNAs encoding various constitutively active $\alpha_{i/o}$-subunits. We found that these constitutively active inhibitory G_α subunits were active on AC-V (inhibiting its activity by 30–80%), but did not inhibit the activity of AC-VIII, suggesting that the effect of $G_{i/o}$PCR on AC-VIII activity is not mediated via these $G_{\alpha i/o}$ subunits.

On the other hand, $G_{\beta 1}$ did inhibit the activity of AC-VIII, and this inhibition was increased in the presence of

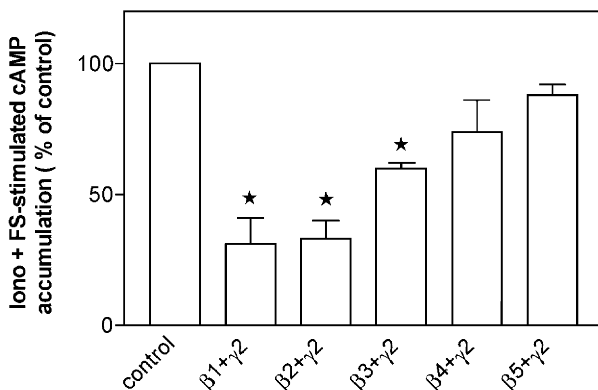

Fig. 6. Effect of different G_β isoforms on AC-VIII activity. COS-7 cells were transfected with 2 μg of AC-VIII cDNA together with 2 μg of $G_{\gamma 2}$ cDNA and 1 μg of the indicated G_β cDNAs. Iono+FS-stimulated cAMP accumulation is expressed as percentage, where 100% is the activity of AC-VIII transfected alone, without G_β and G_γ subunits. ★$p<0.001$ compared with control.

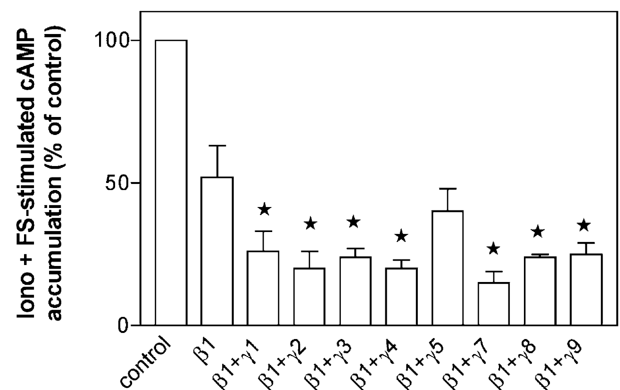

Fig. 7. Effect of different G_γ isoforms on AC-VIII activity. COS-7 cells were transfected with 2 μg of AC-VIII cDNA together with 1 μg of $G_{\beta 1}$ and 2 μg of the indicated G_γ cDNAs. Iono+FS-stimulated cAMP accumulation is expressed as percentage, where 100% is the activity of AC-VIII transfected alone, without $G_{\beta 1}$ and the various G_γ subunits. ★$p<0.01$ compared with β_1.

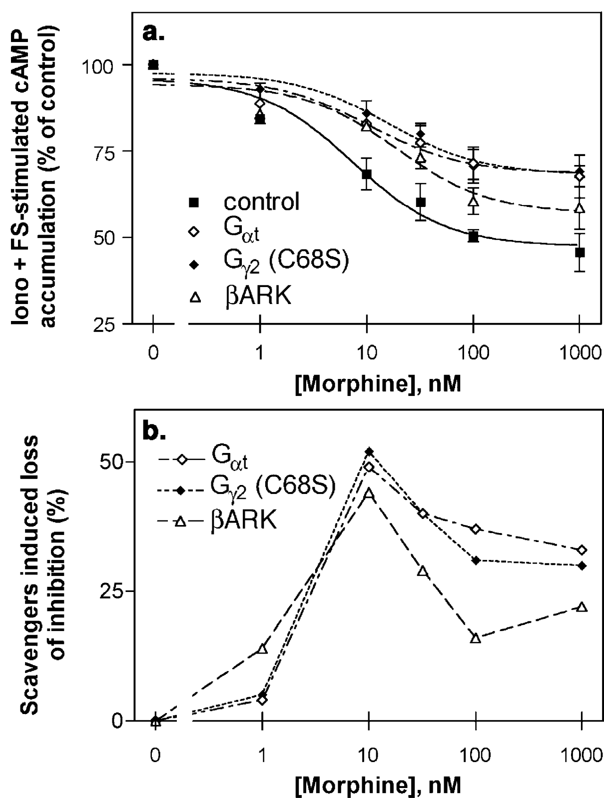

Fig. 8. Inhibition of AC-VIII is attenuated by coexpression of proteins affecting $G_{\beta\gamma}$ activity. COS cells were transfected with 2 μg of AC-VIII and 1 μg μ-opioid receptor cDNAs (control) and where indicated with either $G_{\alpha t}$, $G_{\gamma 2}$(C68S) or CD8-βARK cDNAs (2 μg each). AC-VIII was stimulated with iono+FS. (a) The figure shows the effect of the indicated concentrations of morphine on AC-VIII activity. 100% denotes AC activity in the absence of morphine. (b) The figure shows the effect of scavengers on the morphine-induced inhibition with 100% representing complete reversal of inhibition. The data represent the mean±S.E.M. of three experiments.

$G_{\gamma 2}$. This result is consistent with our previous finding that the addition of $G_{\gamma 2}$ together with $G_{\beta 1}$ further inhibits the activity of AC-I, V and VI compared with the effect of $G_{\beta 1}$ alone [36,37]. The finding that $G_{\beta 1}$, even when added without γ subunits, can inhibit AC-VIII (although to a lower extent) can be attributed to the capacity of this subunit to recruit and couple with endogenous G_γ subunits (present in COS-7 cells) to form an active $G_{\beta\gamma}$ dimer [23]. Moreover, it has been shown that transfection with $G_{\beta 1}$ increases the amount of G_γ subunits in the cells [23].

The effect of G_β on AC-VIII activity seems to be G_β isoform dependent, with β_1 and β_2 being much more efficient than β_3 and β_4, and β_5 being completely inefficient. Differences in the effects of various G_β subunits on AC activity have been shown by McIntire et al. [38] for AC-II and AC-I. Moreover, our group has shown that the activities of AC-V and VI were inhibited more efficiently by β_1 than by β_5 [36]. Regarding other effectors, Zhang et al. [29] and Lindorfer et al. [39] showed that β_5 activates PLCβ2 with the same efficiency as β_1, while β_5 was not able to stimulate the mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) pathways [29]. In addition, Arnot et al. [40] showed that the N-type Ca^{2+} channels (previously shown to be inhibited by $G_{\beta\gamma}$ dimers [41]) are differentially modulated by different G_β isoforms, with $G_{\beta 1}$ and $G_{\beta 3}$ being more effective than $G_{\beta 2}$ and $G_{\beta 4}$, and no significant modulation induced by $G_{\beta 5}$.

It has been shown that G_β subunits can couple with a number of G_γ subunits. However, not all the combinations were active to the same degree. For example, when nine combinations of $G_{\beta 1}$ or $G_{\beta 2}$ with $G_{\gamma(1,2,3,5 \text{ or } 7)}$ were tested for their ability to activate various PLCβ isoforms, all dimers could activate the various PLCβ isoforms except for the retinal-specific $G_{\beta 1\gamma 1}$. Likewise, $G_{\beta 1\gamma 1}$ was markedly less effective in stimulating AC type II and inhibiting AC type I than other $G_{\beta\gamma}$ dimer combinations [42,43]. Here, we show that except for $G_{\gamma 5}$, all other G_γ tested (including γ_1) have increased the inhibitory effect of $G_{\beta 1}$. This result suggests that $G_{\beta 1\gamma 5}$ dimer (if produced) does not inhibit AC-VIII activity.

Scavengers of $G_{\beta\gamma}$ were found to be useful in ascertaining the role of $G_{\beta\gamma}$ in various signaling cascades including MAPK and PLCβ activation, oocyte meiosis arrest and stimulating Ca^{2+} signaling via IP_3 receptors [21,44–46]. Transfecting the cells with $G_{\beta\gamma}$ scavengers, we show that the inhibition of AC-VIII by morphine is attenuated, demonstrating the involvement of $G_{\beta\gamma}$ dimers in this process. However, it is interesting to note that the $G_{\beta\gamma}$ scavengers were not able to completely prevent the inhibition of AC-VIII by opioid receptor activation. This remaining inhibition could hint to a strong affinity of $G_{\beta\gamma}$ dimers to AC-VIII, not allowing complete $G_{\beta\gamma}$ removal by the scavengers. In addition, it has been claimed that upon receptor activation $G_{\beta\gamma}$ and G_α subunits do not have to be completely dissociated one from the other to signal their effect [47–49]. The activity of such an active α−βγ heterotrimer will be inhibited by PTX; however, at this time, we do not know how the $G_{\beta\gamma}$ scavengers would affect such a complex. It is interesting to note that also in the hands of several other laboratories, the effect of $G_{\beta\gamma}$ scavengers was not complete (see examples [50,51]).

In summary, we have shown in intact cells that $G_{\beta\gamma}$ dimers inhibit AC-VIII activity and that the inhibition of AC-VIII by $G_{i/o}$PCR agonist likely results from the effect of $G_{\beta\gamma}$ dimers released upon receptor activation. Further studies will be required to determine if the $G_{\beta\gamma}$ dimer-mediated inhibition of AC-VIII is direct or results from recruitment of other signaling cascades.

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References

[1] L. Birnbaumer, J. Abramowitz, A.M. Brown, Biochem. Biophys. Acta 1031 (1990) 163.
[2] T.M. Cabrera-Vera, J. Vanhauwe, T.O. Thomas, M. Medkova, A. Preininger, M.R. Mazzoni, H.E. Hamm, Endocr. Rev. 24 (2003) 765.
[3] S. Klein, H. Reuveni, A. Levitzki, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3219.
[4] W.-J. Tang, J.A. Iñiguez-Lluhi, S. Mumby, A.G. Gilman, Cold Spring Harbor Symp. Quant. Biol. 57 (1992) 135.
[5] J. Hanoune, N. Defer, Annu. Rev. Pharmacol. Toxicol. 41 (2001) 145.
[6] W.F. Simonds, Trends Pharmacol. Sci. 20 (1999) 66.
[7] R.K. Sunahara, R. Taussig, Mol. Interv. 2 (2002) 168.
[8] N. Defer, M. Best-Belpomme, J. Hanoune, Am. J. Physiol. Renal Physiol. 279 (2000) F400.
[9] K. Iwatsubo, T. Tsunematsu, Y. Ishikawa, Expert Opin. Ther. Targets 7 (2003) 441.
[10] R.K. Sunahara, C.W. Dessauer, A.G. Gilman, Annu. Rev. Pharmacol. Toxicol. 36 (1996) 461.
[11] D.M. Cooper, N. Mons, K. Fagan, Cell. Signal. 6 (1994) 823.
[12] J. Krupinski, F. Coussen, H.A. Bakalyar, W.-J. Tang, P.G. Feinstein, K. Orth, C. Slaughter, R.R. Reed, A.G. Gilman, Science 244 (1989) 1558.
[13] J. Krupinski, J.J. Cali, Adv. Second Messenger Phosphoprot. Res. 32 (1998) 53.
[14] J.J. Cali, J.C. Zwaagstra, N. Mons, D.M.F. Cooper, J. Krupinski, J. Biol. Chem. 269 (1994) 12190.
[15] D.M. Cooper, Biochem. Soc. Trans. 31 (2003) 912.
[16] C. Gu, D.M. Cooper, J. Biol. Chem. 274 (1999) 8012.
[17] T. Avidor-Reiss, I. Nevo, D. Saya, M. Bayewitch, Z. Vogel, J. Biol. Chem. 272 (1997) 5040.
[18] I. Nevo, T. Avidor-Reiss, R. Levy, M. Bayewitch, E. Heldman, Z. Vogel, Mol. Pharmacol. 54 (1998) 419.
[19] M.-H. Rhee, M.L. Bayewitch, T. Avidor-Reiss, R. Levy, Z. Vogel, J. Neurochem. 71 (1998) 1525.
[20] R.C. Thompson, A. Mansour, H. Akil, S.J. Watson, Neuron 11 (1993) 903.
[21] P. Crespo, M.T. Gonzalez-Cachero, N. Xu, J.S. Gutkind, J. Biol. Chem. 270 (1995) 25259.
[22] A. Garritsen, W. Simonds, J. Biol. Chem. 269 (1994) 24418.
[23] W.F. Simonds, J.E. Butrysnki, N. Gautam, C.G. Unson, A.M. Spiegel, J. Biol. Chem. 266 (1991) 5363.
[24] T. Avidor-Reiss, I. Nevo, R. Levy, T. Pfeuffer, Z. Vogel, J. Biol. Chem. 271 (1996) 21309.
[25] A. Goldstein, A. Naidu, Mol. Pharmacol. 36 (1989) 265.
[26] G.B. Downes, N. Gautam, Genomics 62 (1999) 544.
[27] A. Federman, B. Conkiln, K. Schrader, R. Reed, H. Bourne, Nature 356 (1992) 159.
[28] A. Garritsen, P. Gallan, W. Simonds, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 7706.
[29] S. Zhang, O. Coso, C. Lee, J. Gutkind, W. Simonds, J. Biol. Chem. 271 (1996) 33575.
[30] W.J. Koch, B.E. Hawes, J. Inglese, L.M. Luttrell, R.J. Lefkowitz, J. Biol. Chem. 269 (1994) 6193.
[31] M.D. Nielsen, G.C.K. Chan, S.W. Poser, D.R. Storm, J. Biol. Chem. 271 (1996) 33308.
[32] H. Ammer, T.E. Christ, J. Neurochem. 83 (2002) 818.
[33] T. Gudermann, F. Kalkbrenner, G. Schultz, Annu. Rev. Pharmacol. Toxicol. 36 (1996) 429.
[34] S.F. Law, K. Yasuda, G.I. Bell, T. Reisine, J. Biol. Chem. 268 (1993) 10721.
[35] R. Taussig, W.J. Tang, J.R. Hepler, A. Gilman, J. Biol. Chem. 269 (1994) 6093.
[36] M.L. Bayewitch, T. Avidor-Reiss, R. Levy, T. Pfeuffer, I. Nevo, W.F. Simonds, Z. Vogel, FASEB J. 12 (1998) 1019.
[37] M.L. Bayewitch, T. Avidor-Reiss, R. Levy, T. Pfeuffer, I. Nevo, W.F. Simonds, Z. Vogel, J. Biol. Chem. 273 (1998) 2273.
[38] W.E. McIntire, G. MacCleery, J.C. Garrison, J. Biol. Chem. 276 (2001) 15801.
[39] M.A. Lindorfer, C.-S. Myung, Y. Savino, H. Yasuda, R. Khazan, J.C. Garrison, J. Biol. Chem. 273 (1998) 34429.
[40] M.I. Arnot, S.C. Stotz, S.E. Jarvis, G.W. Zamponi, J. Physiol. 527 (2000) 203.
[41] W.A. Catterall, Annu. Rev. Cell Dev. Biol. 16 (2000) 521.
[42] J. Iñiguez-Lluhi, M. Simon, J. Robishaw, A. Gilman, J. Biol. Chem. 267 (1992) 23409.
[43] N. Ueda, J. Iniguez-Lluhi, E. Lee, A. Smrcka, J. Robishaw, A. Gilman, J. Biol. Chem. 269 (1994) 4388.
[44] P. Crespo, N. Xu, W. Simonds, J. Gutkind, Nature 369 (1994) 418.
[45] Y. Sheng, M. Tiberi, R.A. Booth, C. Ma, X.J. Liu, Curr. Biol. 11 (2001) 405.
[46] W. Zeng, D.O. Mak, Q. Li, D.M. Shin, J.K. Foskett, S. Muallem, Curr. Biol. 13 (2003) 872.
[47] M. Bunemann, M. Frank, M.J. Lohse, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 16077.
[48] A. Levitzki, S. Klein, Chembiochem 3 (2002) 815.
[49] R. Rebois, D. Warner, N. Basi, Cell. Signal. 9 (1997) 141.
[50] Y. Blumenstein, O.P. Maximyuk, N. Lozovaya, N.M. Yatsenko, N. Kanevsky, O. Krishtal, N. Dascal, J. Physiol. 556 (2004) 121.
[51] P. Xie, D.D. Browning, N. Hay, N. Mackman, R.D. Ye, J. Biol. Chem. 275 (2000) 24907.