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The (*R*)-enantiomer of CE3F4 is a preferential inhibitor of human exchange protein directly activated by cyclic AMP isoform 1 (Epac1)

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Abbreviations:

007, 8-(4-chloro-phenylthio)-2′-O-methyl-cAMP; CE3F4, 6-Fluoro-5,7-dibromo-2-methyl-1-formyl-1,2,3,4-tetrahydroquinoline; CNB, cyclic nucleotide binding; DEP, Disheveled/Egl-10/Pleckstrin; Epac, exchange protein directly activated by cyclic AMP; GEF, guanine-nucleotide exchange factor; PKA, protein kinase A.

Abstract

Isoform 1 and isoform 2 of exchange protein directly activated by cAMP (Epac1 and Epac2) contribute to cAMP signaling in numerous cellular processes. Their guanine-nucleotide exchange factor (GEF) activity toward the small GTP-binding protein Rap1 is stimulated by the agonist cAMP. CE3F4, a tetrahydroquinoline analog, prevents Epac1 activation *in vitro* and in living cultured cells by inhibiting the GEF activity of Epac1. However, the activity of the (*R*)- and (*S*)-enantiomers of CE3F4, as well as the ability of CE3F4 and its analogs to inhibit Epac2 GEF activity, have not yet been investigated. In this study, we report that (*R*)-CE3F4 is a more potent cAMP antagonist than racemic CE3F4 and (*S*)-CE3F4, inhibiting the GEF activity of Epac1 with 10-times more efficiency than (*S*)-CE3F4. Epac2, in contrast to Epac1, is activated more efficiently by cAMP than by 8-(4-chlorophenylthio)-2'-O-methyladenosine-3',5'-cyclic monophosphate (007), an Epac-selective cAMP analog. (*R*)-CE3F4 displays Epac isoform preference, with 10-fold selectivity for Epac1 over Epac2. Deletion of the N-terminal cyclic nucleotide-binding domain of Epac2 does not affect the characteristics of activation of Epac2 by cAMP and by 007, nor its inhibition by CE3F4. Finally, the evaluation of a series of CE3F4 structural analogs as GEF inhibitors allowed identifying structural features that are important for high Epac1 inhibitory activity of CE3F4. We conclude that the (*R*)-enantiomer of CE3F4 is a preferential inhibitor of Epac1 with high potency in the low micromolar range, and we suggest that this compound may be a useful pharmacological tool for investigating the functional role of Epac1 in cAMP signaling.

Keywords:
cAMP signaling, Epac inhibitor, Rap1 GEF, tetrahydroquinoline analogs, drug enantiomers.

1. Introduction

The second messenger cAMP is involved in the regulation of a number of physiological and pathological processes. Protein kinase A (PKA) and exchange protein directly activated by cyclic AMP (Epac) are two intracellular receptors that transduce cAMP signals [1]. Epac is a Rap1 guanine-nucleotide exchange factor (GEF) for the GTPases Rap1 and Rap2 [2]. There are two isoforms of Epac, Epac1 and Epac2, whose catalytic domain is conserved and the regulatory domain is homologous. However, Epac1 has a single cyclic nucleotide-binding (CNB) domain, whereas Epac2 has two CNB domains, called CNB-A and CNB-B, which are located on both sides of the Disheveled/Egl-10/Pleckstrin (DEP) domain [3]. The additional N-terminal CNB domain in Epac2 has a low affinity for cAMP, and its deletion does not affect the regulation of Epac2 in response to agonists [2]. PKA and Epac can act in synergy or in opposition on downstream effectors and cellular responses, so that pharmacologic agents are essential to discriminate between these two signaling pathways. PKA inhibitors, such as H89 and KT 5720, are widely used to investigate PKA-dependent actions, but these compounds are also known to interfere with a number of other protein kinases and signaling molecules independently of their effects on PKA [4]. 8-(4-chloro-phenylthio)-2′-O-methyl-cAMP, also known as 007, is a cAMP analog which activates Epac, but not PKA, both *in vitro* and *in vivo* [5]. 007 has been widely used to dissect Epac-dependent *versus* PKA-dependent signaling pathways. However, 007 behaves as an inhibitor of several cAMP- and cGMP-phosphodiesterases, which may indirectly result in increased intracellular concentrations of cAMP and cGMP [6]. It is therefore crucial to develop specific Epac inhibitors.

We recently identified a tetrahydroquinoline analog named CE3F4 that inhibits Epac1 GEF activity toward its effector Rap1 both in cell-free systems and in intact cells, and has no influence on PKA activity [7]. The kinetic properties of CE3F4, which has an asymmetric carbon atom and thus two enantiomers, were initially characterized using the racemic mixture. In the present study, we isolated the two CE3F4 enantiomers to explore their respective properties relative to Epac1 activity. Next, we aimed at determining whether CE3F4 possesses selectivity between Epac1 and Epac2. To this end, we have produced functional recombinant Epac2 proteins deleted - or not - of the N-terminal CNB-A domain to study their guanine nucleotide exchange activity toward Rap1. Then, we have compared the effects of CE3F4 and its analogs on recombinant Epac2 and Epac1 exchange activity.

2. Material and methods

2.1. Reagents

8-(4-chlorophenylthio)-2'-O-methyladenosine-3',5'-cyclic monophosphate (007), was from Biolog, Bremen, Germany, BODIPY FL 2'-(or-3')-*O*-(*N*-(2-aminoethyl)urethane), bis(triethylammonium) salt (Bodipy-GDP) was from Invitrogen. 6-Fluoro-5,7-dibromo-2-methyl-1-formyl-1,2,3,4-tetrahydroquinoline (CE3F4) and related compounds [a], [b], [c], [d], [g], and [h] (see structures in Fig 3C) were synthesized according to methods published previously [8]. 6-Fluoro-5,7-dibromo-1-formyl-1,2,3,4-tetrahydroquinoline (2-demethyl-CE3F4, compound [f]) was synthesized from 6-fluoroquinoline by reduction and formylation of this compound to give 6-fluoro-1-formyl-1,2,3,4-tetrahydroquinoline, which was then brominated to give 2-demethyl-CE3F4. 5,7-dibromo-1-formyl-2-methyl-1,2,3,4-tetrahydroquinoline (compound [e]) was synthesized from 3,5-dibromoaniline by a Skraup synthesis to give 5,7-dibromoquinaldine followed by reduction of nitrogen-containing ring and formylation of nitrogen in position 1. The (*R*)-and (*S*)-enantiomers of CE3F4 were derived from the (*R*)- and (*S*)-enantiomers of 6-fluoro-2-methyl-1,2,3,4-tetrahydroquinoline (6-FTHQ). (*R*)- and (*S*)-6-FTHQ were obtained according to methods published previously [9] and were then formylated and brominated to give, respectively, (*R*)-CE3F4 and (*S*)-CE3F4. The mass spectra and nuclear magnetic resonance of these compounds were identical to those reported previously for racemic CE3F4 **(**Bouyssou P., 1990. Contribution à l'étude des quinolones carboxyliques de structure type Benzo [i,j] Quinolizine, relation structure activité. Ph.D. Thesis. Orléans University, France). Optical rotation measurements were performed at 589 nm, 20°C and c = 10 mg/ml in chloroform, with an accuracy of ± 1°, using a Perkin Elmer model 341 polarimeter.

2.2. Recombinant protein expression

Protein NCBI Reference Sequences for human Epac1 and Epac2 are NP_006096 and NP_008954, respectively. Recombinant human Epac1 (residues 149–881), deleted of its Dishevelled, Egl-10, and Pleckstrin (DEP) domain, and human Rap1A were produced with GST as a fusion tag, as described previously [7]. Human Epac2(AB) (amino acids 43-1011) carries both the CNB-A and the CNB-B domains. Human Epac2(B) (amino acids 283-1011) lacks the first 283 amino acids of Epac2. It is therefore deleted of its CNB-A and DEP

domains, but retains the CNB-B domain and the full catalytic region. Epac2(AB) was obtained by HindIII/NotI restriction and Epac2(B) was obtained by Ssp1/Not1 restriction of a human Epac2A cDNA (a gift from Ann M Graybiel, Department of Brain and Cognitive Sciences, MIT, Cambridge). Both cDNA fragments were inserted into pET41a (Novagen), expressed in *Escherichia coli* Rosetta 2(DE3) (Novagen), and the GST-tagged fusion proteins were purified by nickel-nitrilotriacetic acid beads (Qiagen), as described previously for Epac1 [7].

2.3. Assay of in vitro GEF activity

In vitro GDP exchange catalyzed by Epac was measured using purified recombinant Epac isoforms and Rap1A loaded with Bodipy-GDP, as described previously [7]. The GEF activity of Epac proteins was initiated by injection of the agonist. Stock solutions of CE3F4 and its analogs (12 mM in 100% DMSO) were kept at -20 °C and were diluted in assay buffer (0.67 % final DMSO concentration) just before use for GDP exchange assays. The release of Bodipy-GDP was measured in real time as the decay of fluorescence. A single exponential was fit to the time-course data using the Graphpad Prism program. The initial velocity (Vi) of GDP exchange on Rap1 was calculated as described previously [7].

2.4. Data analysis

Data are expressed as mean ± S.D. Differences in quantitative variables were examined by unpaired one- or two-tailed Student's *t* test. EC_{50} and IC_{50} values were computed according to a four-parameters dose-response model and compared on the basis of the extra sum-of square F test, using Graphpad Prism. Two-tailed Wilcoxon matched-pair signed rank tests were performed using GraphPad Prism.

3. Results

3.1. (R)-CE3F4 is a more potent inhibitor of cAMP-activated Epac1 than (S)-CE3F4

CE3F4 is a chiral molecule, composed of the (*S*)- and (*R*)-enantiomers in equal parts, whose formulas are given in Fig 1A. The individual enantiomers, (*R*)-CE3F4 and (*S*)-CE3F4, were obtained as described in Materials and methods. (*R*)-CE3F4 is levorotatory with [α]D =

-12 ± 1°, while (*S*)-CE3F4 is dextrorotatory with [α]D = +11 ± 1°. Fig 1B shows a representative dose-response inhibition assay in which the GEF activity of 007-activated Epac1 toward Rap1 was measured in the presence of increasing concentrations of racemic CE3F4 or of its individual enantiomers (*R*)-(-)-CE3F4 and (*S*)-(+)-CE3F4. The dose-response curve for the (*R*)-enantiomer is significantly (F test) left-shifted relative to that obtained with the racemate, whereas the (*S*)-enantiomer behaves only as a weak inhibitor.

We have synthesized 2-demethyl-CE3F4 (Fig 1A), which does not have a stereocenter. The dose-response curve obtained with 2-demethyl-CE3F4 (Fig 1B) was not significantly different (F test) from the one obtained with (*S*)-CE3F4. The IC_{50} values for the racemate, enantiomers and demethyl analog were determined several times independently (Fig 1C). (*R*)-CE3F4 inhibited Epac1 GEF activity with an IC_{50}, which was ~2-fold smaller than that obtained with the racemic CE3F4, and ~10-fold smaller that the IC_{50} values of (*S*)-CE3F4 and demethyl-CE3F4.

3.2. cAMP and 007 differentially activate Epac1 and Epac2

In order to define the appropriate experimental conditions for comparing the inhibitory properties of CE3F4 and its structural analogs against Epac1 and Epac2 GEF activities, the activation of the recombinant proteins was studied using 007 or cAMP as agonists. The GEF activities of Epac1, Epac2(AB) that possesses both the CNB-A and the CNB-B domains, or Epac2(B) that is deleted of its CNB-A domain, were measured in the presence of either 50 μM 007 or 300 μM cAMP. These concentrations of cyclic nucleotides were known from previous studies [7] to give near-maximal activation of Epac1. Fig 2A shows that 007 was a stronger activator of Epac1 than cAMP, inducing an almost 30-fold activation over basal. Conversely, 007 was about half less active than cAMP in activating Epac2(AB) and Epac2(B). Dose-response studies were performed (Figs 2B and 2D) to determine the effective concentrations that produced 50% of the maximum response (EC_{50}) and the maximal GEF activities at saturating concentrations of agonists (V_{max}). The values are summarized in Fig 2C (means from two independent dose-response experiments). The apparent affinity of Epac1 was 6 times higher for 007 than for cAMP and the V_{max} of 007-activated Epac1 was 5-fold larger than the V_{max} of cAMP-activated Epac1. In contrast, the apparent affinity of Epac2(B) was 50% higher for cAMP than for 007 and the V_{max} of cAMP-activated Epac2(B) was 40% larger than the V_{max} of 007-activated Epac2(B). Consequently, the apparent affinity of Epac2(B) for cAMP activation was 15-fold higher than that of Epac1.

3.3. (R)-CE3F4 and its analogs differentially inhibit Epac1 and Epac2

Epac1, Epac2(AB) and Epac2(B), were activated by saturating concentrations of either 007 (50 μM) or cAMP (300 μM). Fig. 3A shows that, whatever the agonist used, the GEF activity of Epac1 was much more inhibited by 50 μM of racemic CE3F4 than the GEF activity of Epac2(AB) or Epac2(B). On the other hand, there was no significant difference in the moderate inhibitory effects of racemic CE3F4 on the GEF activity of Epac2(AB) or of Epac2(B). (*R*)-CE3F4 was then used to study the concentration-dependent inhibition of the GEF activities of Epac1 and Epac2(B) activated by cAMP (300 μM). Fig. 3B shows that the dose-response curve obtained with Epac2(B) is strongly right-shifted relative to that obtained with Epac1. From two independent dose-response experiments such as the one shown in Fig 3B, the mean IC_{50} of (*R*)-CE3F4 was 4.2 μM for Epac1 and 44 μM for Epac2(B).

cAMP-activated Epac1 and Epac2(B) were compared for their ability to be inhibited by several CE3F4 structural analogs. The structural characteristics of these analogs, lettered from [a] through [h], are summarized in Figure 3C. All of them are in the racemic form, except for the non-chiral analog 2-demethyl-CE3F4 (compound [f]). Figure 3D shows the results of a representative experiment, expressed as the relative inhibitory potencies of the various analogs (30 μM), measured in the presence of cAMP (300 μM) as the agonist. Without considering compound [d], which had no significant impact on both Epac isoforms, the analogs were consistently weaker cAMP antagonists toward Epac2(B) than toward Epac1 by two-tailed Wilcoxon matched-pair signed rank test (p value < 1 %,). In fact, each of these compounds taken individually was significantly less potent to inhibit Epac2(B) than to inhibit Epac1 (p value < 5%, one-tailed Student's t-test). A second independent experiment (not shown) yielded highly similar results in terms of rank order of relative inhibitory potencies toward the two Epac isoforms. The stronger antagonist of Epac1 and Epac 2(B) activation by cAMP was CE3F4 itself. A dose-response study (not shown) indicated that the IC_{50} of racemic CE3F4 was 11 μM for Epac1 and 66 μM for Epac2(B) when they were activated by 300 μM cAMP. Compound [a], which lacks bromine on position 7, compound [e], which lacks fluorine on position 6, compound [f] (2-demethyl-CE3F4), compound [g], in which an acetyl group is substituted on position 1 for the formyl group present in CE3F4, and compound [h], which lacks the formyl group, had reduced antagonistic properties toward both Epac isoforms. Finally, compound [b], which lacks bromine on position 7, and compounds [c] and [d], that no longer possess bromine atoms on both positions 5 and 7, showed only little

inhibitory activity.

4. Discussion

In the present study, we report that the inhibition of Epac exchange activity by CE3F4 is enantioselective, and that CE3F4 preferentially inhibits the GEF activity of Epac1 versus that of Epac2. Therefore, our data demonstrate ligand specificity among CE3F4 analogs, and Epac isoform specificity for CE3F4 inhibition of GEF activity toward Rap1. Truncated mouse Epac2, deleted from the first CNB domain (CNB-A) and the DEP domain, was previously used successfully to study the conformational changes induced by cAMP [10], indicating that the second CNB domain (CNB-B) of Epac2 is sufficient to promote full cAMP dependence of GEF activity. In the present work, we have also checked that Epac2 showed similar characteristics of activation by agonists, whether or not the CNB-A domain was deleted. Furthermore, we have verified that the weak antagonistic effect of CE3F4 was similar using Epac2(AB) or Epac2(B). Therefore, Epac2(B), which can be produced at higher yields than Epac2(AB), was preferentially used for further studies of the mechanisms of activation of Epac2 by cyclic nucleotides and of inhibition of its GEF activity by CE3F4 and its analogs.

007 is widely used to activate Epac proteins in intact cells [11, 12], because substitution of the 2'-hydroxyl group of cAMP by a 2'-O-methyl group allows 007 to selectively activate Epac over PKA [5]. 007 is much more efficient than cAMP in activating recombinant Epac1 *in vitro* [13], so that this analog was called a superagonist of Epac1. We found here that cAMP was a better agonist than 007 to activate Epac2 *in vitro*, with a lower EC_{50} and a higher maximal GEF activity under saturating conditions. Even so, CE3F4 was a better inhibitor of Epac1 than of Epac2, independently of which agonist was used as an activator.

Since marked differences can exist in the pharmacological profile of drug enantiomers [14], the *(R)* and (*S*) enantiomers of CE3F4 were synthesized with the aim of studying their inhibitory potency and selectivity. The dose-response studies show that the (*R*)-enantiomer was 10 times more active than its (*S*) counterpart in inhibiting 007-activated Epac1 GEF activity. This result indicates that Epac1 provides a chiral molecular environment that strongly distinguishes between the two enantiomers of CE3F4, and this further strengthens the specific nature of the interaction between CE3F4 and Epac1. The (*R*) configuration of the 2-methyl group of CE3F4 may favor the interaction between CE3F4 and agonist-activated Epac1, or, alternatively, the (*S*) configuration may have a negative impact on this interaction. The first hypothesis is strongly supported by the fact that 2-demethyl-CE3F4, that lacks the

methyl group on the chiral carbon 2 of CE3F4, behaved as a poor inhibitor, with an IC_{50} similar to that of the (*S*)-enantiomer.

The detailed dose-response studies showed that (*R*)-CE3F4 blocked cAMP-dependent Epac1 GEF activity with a low micromolar potency and a 10-fold preference for Epac1 versus Epac2. Among the racemic CE3F4 analogs tested, none had an improved activity toward Epac2 compared to (*R*)-CE3F4, that remains the least bad Epac2 antagonist. Several substituents are major determinants of the antagonistic properties of CE3F4 analogs toward cAMP-activated Epac1: bromine at positions 5 and 7, fluorine at position 6, formyl at position 1, and methyl at position 2. These structure-activity studies, including the importance of the spatial orientation of the methyl group at position 2, indicate that the binding site of CE3F4 on Epac1 is highly specific.

Several other Epac1 and Epac2 small-molecule inhibitors have been recently identified, on the basis of their ability to compete with a fluorescent derivative of cAMP [15]. Some of them (designated as ESI-05 and ESI-07) inhibited cAMP-mediated Epac2 GEF activity in the submicromolar range but were quite ineffective in suppressing Epac1 GEF activity [16]. Others, such as ESI-09, inhibited both Epac1 and Epac2 GEF activities in the low micromolar range [17]. These compounds are all chemically unrelated to CE3F4 and its analogs, and their mechanisms of action are most likely different. Indeed, contrary to ESI compounds, CE3F4 and its analogs do not directly compete with cAMP. Although the detailed molecular mechanisms remain to be elucidated, the inhibition is of the uncompetitive type [7], so that they have no effect on the constitutive GEF activity of Epac (i.e. in the absence of agonist) and their antagonistic potency increases when the concentration of agonist increases. This mode of inhibition may be of critical interest in pathophysiological situations such as chronic heart failure, which is associated with an elevated level of catecholamines and an overstimulation of the adenylyl cyclase-cAMP system in cardiomyocytes, or in the subsarcolemmal space of various cell types, including cardiomyocytes, in which local cAMP levels can reach high micromolar concentrations [18].

In summary, our results indicate that (*R*)-(-)-CE3F4 is a more potent Epac1 antagonist than (*S*)-(+)-CE3F4, as demonstrated by its greater inhibition of in vitro Epac1 GEF activity for the small GTP binding protein Rap1. In addition, we show that (*R*)-CE3F4 displays Epac isoform preference, with a 10-fold selectivity for Epac1 over Epac2. Finally, the biochemical evaluation of a series of tetrahydroquinoline analogs as GEF inhibitors allowed identifying structural features that are important for high Epac1 inhibitory activity of CE3F4. We conclude that the (*R*)-enantiomer of CE3F4 may be a useful pharmacological tool for

investigating the functional role of Epac proteins.

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Figure captions.

Fig. 1. (*R*)-CE3F4 is the active enantiomer that antagonizes Epac1 activation. (A) Chemical structure of CE3F4 analogs used as inhibitors of Epac1 GEF activity. (B). Initial velocities of GEF activity of Epac1 activated by 20 μM 007 were measured in the presence of increasing concentrations of racemic CE3F4 (empty circles), (*R*)-stereoisomer of CE3F4 (black squares), (*S*)-stereoisomer of CE3F4 (black diamonds), and 2-demethyl-CE3F4 (empty triangles). Each experimental point is the mean of initial velocity values computed from triplicate time-course experiments. IC_{50} values were computed using Graphpad Prism, according to a four-parameters dose-response model. (C) Mean ± SD of n = 4 to 9 independent determinations of IC_{50} values for each inhibitor.

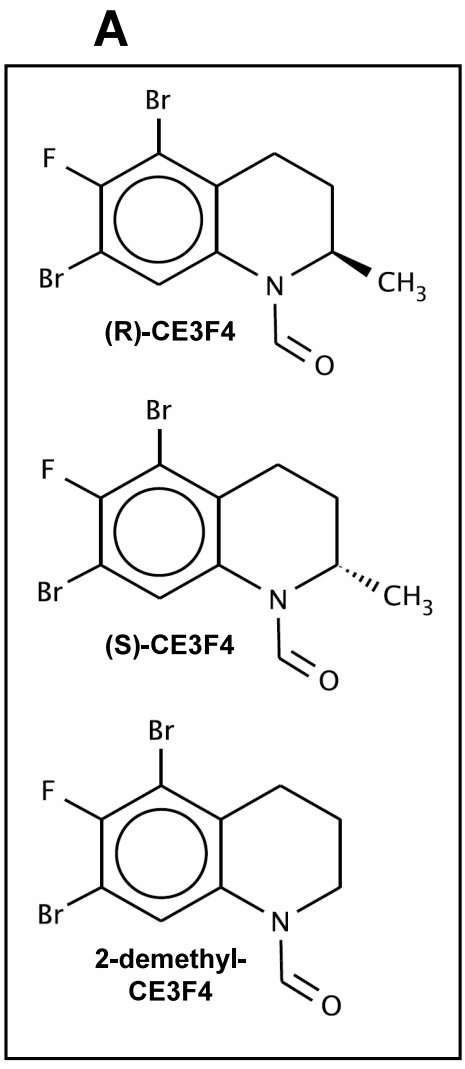
Fig. 2. Differential activation of Epac1 and Epac2 by cAMP and 007

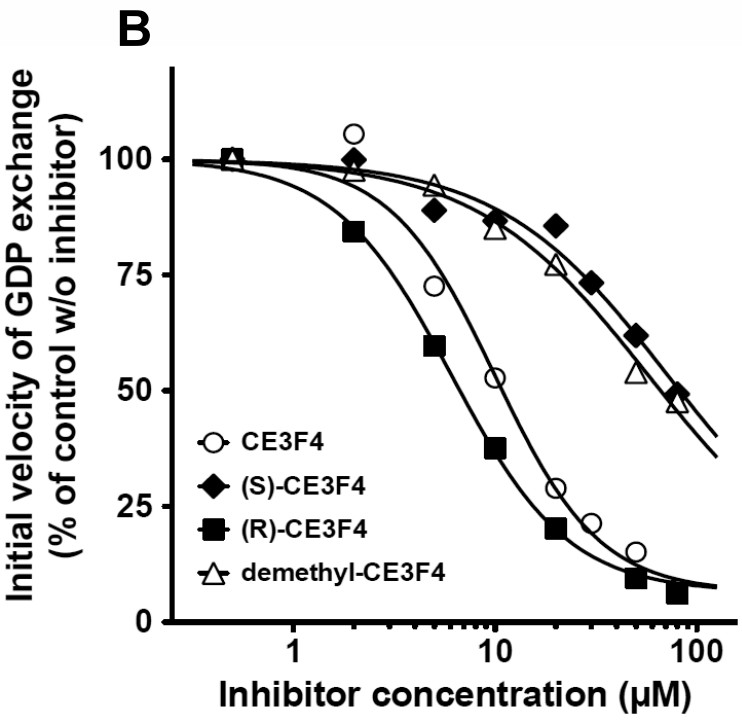
The initial velocity of GDP exchange catalyzed by Epac1, Epac2(AB), which carries both the CNB-A and the CNB-B domains, or Epac2(B), which is devoid of the CNB-A domain, was measured in triplicate in the presence or absence of 50 μM 007 or 300 μM cAMP. The agonist-dependent activity (mean ± S.D., n = 3) was obtained after subtraction of the constitutive GDP exchange activity, measured in the presence of Epac proteins but in the absence of agonist. (A) Epac1, Epac2(AB), and Epac2(B), as indicated in the figure, were incubated without agonist (Ctrl, empty bars), or with 007 (hatched bars) or cAMP (black bars). Results are expressed as fold activation relative to Ctrl. *, $p < 1\%$ comparing one agonist versus the other. (B, D) Epac1 (panel B) or Epac2B (panel D) were activated by increasing concentrations of 007 (empty squares) or cAMP (black circles). The initial velocity of GDP exchange (mean ± S.D. of triplicates) was plotted against the log of agonist (cAMP or 007) concentration. $p < 0.01\%$ by comparison of fits (EC_{50} and maximal velocity of exchange) based of the extra sum-of square F test (Graphpad Prism). (C) Summary of EC_{50} values and maximal velocities of exchange (V_{max}) (means from two independent dose-response experiments such as those shown in (B) and (D)).

Fig. 3. Preferential inhibition of Epac1 by CE3F4 and its analogs. The initial velocity of GDP exchange catalyzed by Epac1, Epac2(AB), or Epac2(B), was measured in triplicate in the presence or absence of agonists and of inhibitory compounds. The agonist-dependent activity was obtained by subtraction of the constitutive GDP exchange activity, measured in the presence of Epac proteins but in the absence of agonists and inhibitors. Results are shown

as mean ± S.D. (A) Epac1 (black bars), Epac2(AB) (empty bars), and Epac2(B) (hatched bars) were incubated with 50 µM racemic CE3F4 and with 50 µM 007 or 300 µM cAMP, as indicated below the x-axis. Results are expressed as the % of the initial velocity of GDP exchange measured in the absence of racemic CE3F4. *, $p < 1\%$; ns (not significant), $p > 5\%$ by two-tailed Student's t-test. (B) Epac1 (black circles) or Epac2(B) (empty squares) were activated by 300 µM cAMP, and the initial velocity of GDP exchange was measured in triplicate in the presence of increasing concentrations of (*R*)-CE3F4. Results are expressed as the % of the initial velocity of GDP exchange measured in the absence of (*R*)-CE3F4. $p < 0.1\%$ by comparison of fits (IC_{50}) based of the extra sum-of square F test (Graphpad Prism). (C) Chemical structure of analogs of CE3F4 identified as compounds [a] to [h]. Numbering of the positions are that of the tetrahydroquinoline skeleton. (D) The initial velocity of GDP exchange catalyzed by Epac1 (hatched bars) or Epac2(B) (black bars) was measured in triplicate in the presence of 30 µM racemic CE3F4 or compounds [a]-[h], and 300 µM cAMP as the agonist. Results are expressed relative to the control values measured in the absence of inhibitor, which were set at 100%. $p < 0.01\%$ for global comparison of Epac2(B) and Epac1 (Wilcoxon matched-pair signed rank test). $p < 5\%$ or less for comparison of Epac2(B) and Epac1 to be inhibited by each compound (one-tailed Student's t-test).

Figure 1

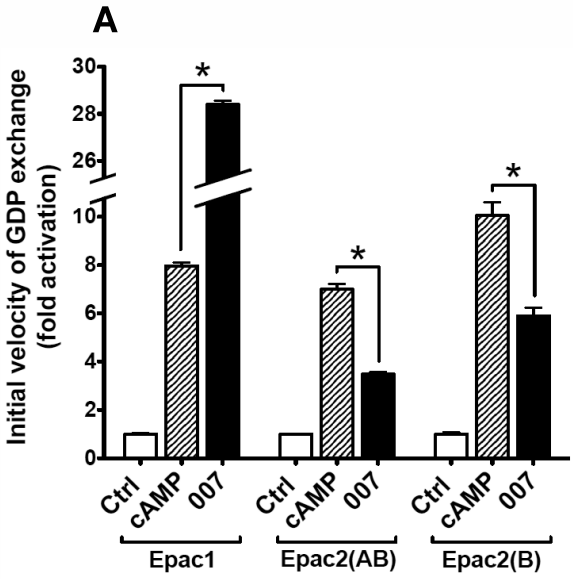


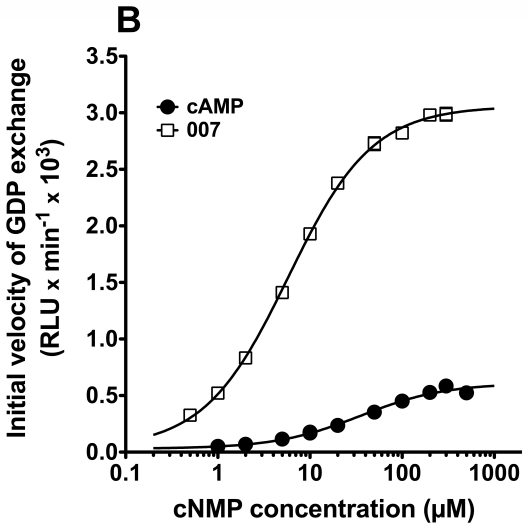


C

	Mean EC50 (μM)	SD	n	p
CE3F4	10.7	1.4	6	< 1%
(R)-CE3F4	5.8	0.8	9	
(S)-CE3F4	56	7	8	NS
Demethyl-CE3F4	50	5	4	

Figure 2





C

EC50 (µM)

	Epac1	Epac2(B)
007	5,6	3,4
cAMP	36	2,3

Vmax (RLU/min/10E-3)

	Epac1	Epac2(B)
007	3,1	0,63
cAMP	0,69	0,97

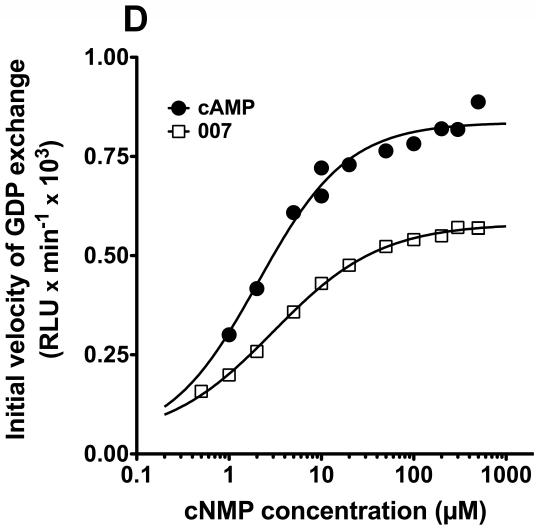
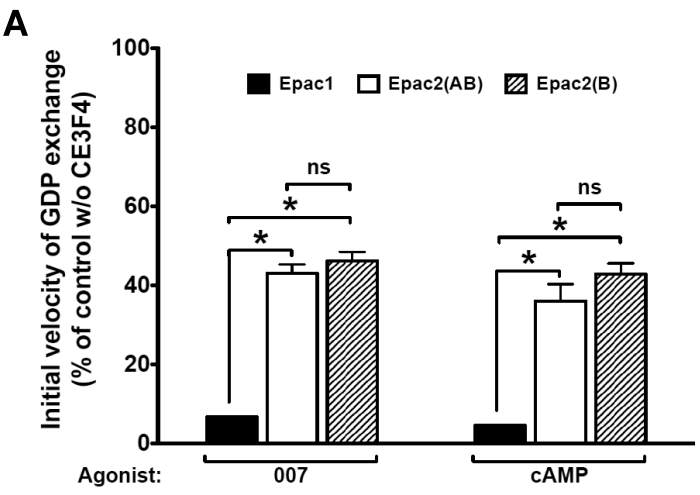
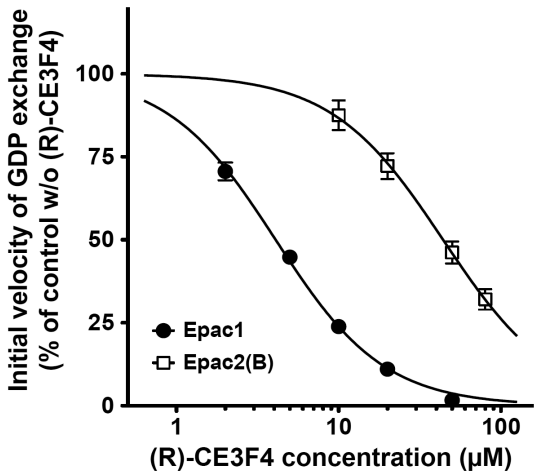


Figure 3





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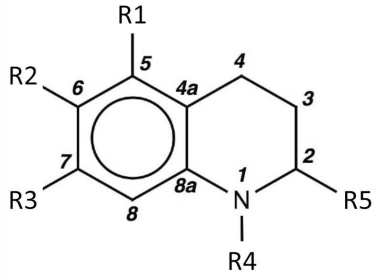
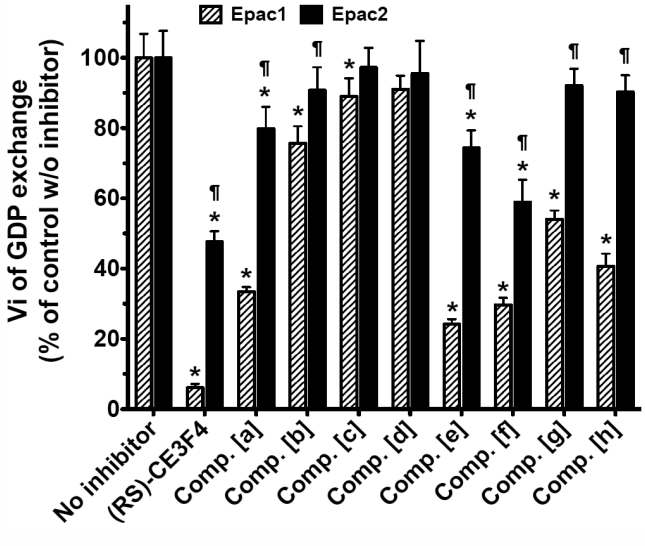
		[a]	[b]	[c]	[d]	[e]	[f]	[g]	[h]
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R2	=	F	F	F	F	H	F	F	F
R3	=	H	Br	Nitrile	H	Br	Br	Br	Br
R4	=	Formyl	Formyl	Formyl	Formyl	Formyl	Formyl	Acetyl	H
R5	=	Methyl	Methyl	Methyl	Methyl	Methyl	H	Methyl	Methyl