Biochemical and Biophysical Research Communications 441 (2013) 387–392

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dynamic modulation of FGFR1–5-HT1A heteroreceptor complexes. Agonist treatment enhances participation of FGFR1 and 5-HT1A homodimers and recruitment of β-arrestin2

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ARTICLE INFO

Article history:
Received 9 October 2013
Available online 21 October 2013

Keywords:
G-protein-coupled receptors
Receptor tyrosine kinases
Fibroblast growth factor receptor 1
Serotonin receptors
Bioluminescence resonance energy transfer
Heterocomplex
Homodimerization
Allosteric modulation
Protein–protein interactions

ABSTRACT

New findings show that neurotrophic and antidepressant effects of 5-HT in brain can, in part, be mediated by activation of the 5-HT1A receptor protomer in the hippocampal and raphe FGFR1–5-HT1A heteroreceptor complexes enhancing the FGFR1 signaling. The dynamic agonist modulation of the FGFR1–5-HT1A heteroreceptor complexes and their recruitment of β-arrestin is now determined in cellular models with focus on its impact on 5-HT1AR and FGFR1 homodimerization in the heteroreceptor complexes based on BRET^2 assays. The findings show that coagonist treatment with 8-OH-DPAT and FGF2 but not treatment with the 5-HT1A agonist alone markedly increases the BRETmax values and significantly reduces the BRET50 values of 5HT1A homodimerization. The effects of FGF2 or FGF20 with or without the 5-HT1A agonist were also studied on the FGFR1 homodimerization of the heteroreceptor complexes. FGF2 produced a marked and rapid increase in FGFR1 homodimerization which partially declined over a 10 min period. Cotreatment with FGF2 and 5-HT1A agonist blocked this decline in FGFR1 homodimerization. Furthermore, FGF2 alone produced a small increase in the BRET^2 signal from the 5-HT1A-β-arrestin2 receptor–protein complex which was additive to the marked effect of 8-OH-DPAT alone. Taken together, the participation of 5-HT1A and FGFR1 homodimers and recruitment of β-arrestin2 was demonstrated in the FGFR1–5-HT1A heteroreceptor complexes upon agonist treatments.

1. Introduction

In 1999 the concept was introduced that the growth-promoting activity of many G protein coupled receptors (GPCRs) involves activation of receptor tyrosine kinases (RTKs) and their downstream signaling cascades [1]. Many observations led to the emergence of the so called 'transactivation' concept, which refers to the activation of RTKs by GPCR ligands and links GPCR signaling to the mitogen activated protein kinase signaling cascade [1–4]. A bidirectional cross-communication between RTKs and GPCRs appears to exist involving e.g. heterotrimeric G proteins and β-arrestins [5–7]. Recently evidence was obtained that integration of neurotrophic factor and transmitter signaling can take place in RTK-GPCR heteroreceptor complexes at the level of the plasma membrane [3,8–10].

New findings show that neurotrophic and antidepressant effects of 5-HT in brain can, in part, be mediated by activation of the 5-HT1A receptor protomer in the hippocampal FGFR1–5-HT1A heteroreceptor complexes enhancing the FGFR1 signaling [9]. The dynamic modulation of these heteroreceptor complexes has been continued in the current study using the BRET^2 assay in HEK293T cells. The participation of 5-HT1A and FGFR1 homodimers and recruitment of β-arrestin2 is demonstrated upon agonist treatment.

2. Materials and methods

Detailed descriptions are available in Supplementary material on: chemicals and reagents; receptor constructs; cell culture,

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http://dx.doi.org/10.1016/j.bbrc.2013.10.067

transfection and immunofluorescence microscopy; membrane preparation and ligand binding assay.

2.1. Quantitative BRET^2 saturation assay

Forty-eight hours after transfection HEK293T cells with constant (0,5 μg) or increasing amounts of cDNA of Receptor-Rluc8 (5-HT1A or FGFR1) and Receptor-GFP^2 (5-HT1A or FGFR1) respectively were rapidly washed twice in PBS, detached, and resuspended in the same buffer. Cell suspensions (20 μg of protein) were distributed in duplicates into 96-well microplates (either black clear-bottomed or white opaque, Corning 3651 or 3600) for fluorescence and luminescence determinations. The total fluorescence of cell suspensions was quantified and then divided by the background (mock-transfected cells) in a POLARstar Optima plate-reader (BMG Lab-technologies, Offenburg, Germany) equipped with a high-energy xenon flash lamp, using a 10 nm bandwidth excitation filter at 400 nm, and 10 nm bandwidth emission filter corresponding to 510 nm. And total bioluminescence was determined on samples incubated for 10 min with 5 μM h-coelenterazine (Molecular Probes, Eugene, OR, USA). The background values for total luminescence were negligible and subtracted from sample values. For BRET^2 measurement, coelenterazine-400a also known as *DeepBlue*™ C substrate (VWR, Sweden) was added at a final concentration of 5 μM, and readings were performed 10 s after each injection using the POLARstar Optima plate-reader (BMG Labtechnologies, Offenburg, Germany) that allows the sequential integration of the signals detected with two filter settings [410 nm (with 80 nm bandwidth) and 515 nm (with 30 nm bandwidth)]. The BRET^2 ratio is defined as previously described [11,12]. For determining the effects of each receptor agonist and the combined treatment, transfected HEK293T cells were incubated for 10 min at 25 °C in the absence or presence of the indicated agonist concentration prior to performing BRET^2 analysis.

2.2. BRET^2 competition assay

Forty-eight hours after transfection, HEK293T cells transiently transfected with constant amounts (0.5 μg) of plasmids encoding for FGFR1^{Rluc8} and 5-HT1A^{GFP2} and increasing amounts (0.1–8 μg) of plasmids encoding for wild-type FGFR1 or 5-HT1A and the mock pcDNA3.1+; respectively. The energy transfer was determined as described for the BRET^2 saturation assay. For further details in titration of donor and acceptor fusion proteins curves see Supplementary materials.

2.3. Monitoring FGFR1 activation using BRET^{2}assay

For concentration–response and kinetic BRET^2 experiments, HEK293T cells were transiently transfected at a constant ratio (1:2:1) of FGFR1^{Rluc8}/FGFR1^{GFP2}/5HT1A in presence of heparin (0.5 μM). Cells were treated with the indicated FGF ligand concentration, in presence or absence of 5-HT1A agonist, or vehicle for 2 min before BRET^2 measurement. FGF ligand-promoted BRET^2 was calculated by subtracting the BRET^2 ratio obtained in the absence of the FGF ligand from that obtained in the presence of the FGF ligand. In kinetic measurements, coelenterazine-400a was injected simultaneously with FGF ligand (30 ng/ml) and 8-OH-DPAT, and reading were then collected 2 s after each injection. In each experiment, the specificities of FGFR1/FGFR1 interactions were assessed by comparison with cells expressing FGFR1^{GFP2} alone. Also as a negative control was used cells individually expressing FGFR1^{Rluc8} mixed prior to exposition to coelenterazine-400a with cells individually expressing FGFR1^{GFP2}.

2.4. Monitoring receptor-interacting proteins (β-arrestin2) using BRET^{2}assay

For dose–response and kinetic BRET^2 experiments, HEK293T cells were transiently transfected at a constant ratio (1:1:2) of FGFR1/5HT1A^{Rluc8}/β-arrestin2^{GFP2} and/or (1:2) for FGFR1^{Rluc8}-β-arrestin2^{GFP2}. Agonist-promoted BRET^2 was calculated by subtracting the BRET^2 ratio obtained in the absence of agonist addition from the one obtained in the presence of an agonist. In the case of kinetic measurements, coelenterazine-400a was injected simultaneously with the agonist, and reading were then collected 2 s after each injection. In each experiment, the specificities of Receptor–β-arrestin2 interactions were assessed by comparison with cells expressing Receptor^{GFP2} alone.

2.5. Statistical analysis

The number of samples (n) in each experimental condition is indicated in Figure legends. All data were analyzed using the commercial program GraphPad PRISM 4.0 (GraphPad Software, USA). When two experimental conditions were compared, statistical analysis was performed using an unpaired t test. Otherwise, statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison post-test. The P value 0.05 and lower was considered significant. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. BRET isotherms were fitted using a nonlinear regression equation assuming a single binding site, which provided BRETmax and BRET50 values. The correlation between fluorescence or luminescence and receptor density was analyzed by a linear regression curve fitting with the same software.

3. Results

The existence of FGFR1–5-HT1A heteroreceptor complexes and their agonist regulation by 8-OH-DPAT and/or FGF2 [9] was demonstrated previously. However, the dynamic modulation of these heteroreceptor complexes was not previously described.

3.1. Effects of combined treatment with the 5-HT1A agonist 8-OH-DPAT and FGF2 on 5-HT1A receptor homodimerization in HEK cells containing FGFR1–5-HT1A heteroreceptor complexes

HEK293T-27 cells were transiently co-transfected with a constant amount of FGFR1 (1 μg cDNA), 5HT1A-Rluc8 (1 μg cDNA), and increasing concentrations of 5-HT1A-GFP2 (0.25–5 μg cDNA) and then treated with the 5-HT1A agonist 8-OH-DPAT and FGF2 or with 8-OH-DPAT alone. A highly significant increase in the quantitative BRET^2 signal was found for the 5-HT1A homodimer when analyzed in the presence of FGF2 (50 ng/ml) and 8-OH-DPAT (100 nM), which gives BRETmax value of 33.78 ± 0.97 (means ± S.E.M., mBU) (Fig. 1A). 8-OH-DPAT (100 nM) alone failed to change the BRETmax value. The combined treatment with FGF2 and 8-OH-DPAT also produced a significant reduction of the BRET50 value indicating an increase in the affinity for the interaction between the two protomers (Fig. 1B).

3.2. Effects of combined and single treatment with 8-OH-DPAT and FGF2 on FGFR1 homodimerization in HEK cells containing FGFR1–5-HT1A heteroreceptor complexes

We have previously demonstrated with BRET^2 analysis that FGF2 increases the formation of FGFR1 homodimers [13]. In the current study the modulatory effect of 5-HT1A agonist 8-OH-DPAT was studied on the FGF2 induced FGFR1/FGFR1 homodimer formation by means of BRET^2 analysis. HEK293T cells were transiently

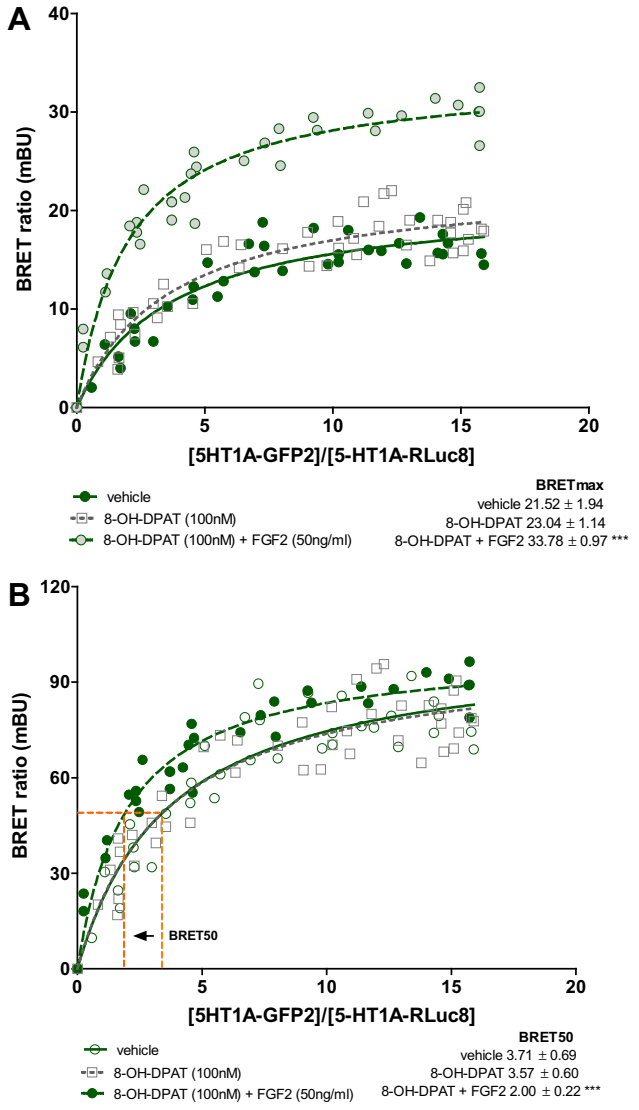

Fig. 1. Quantitative BRET^2 analysis shows the dynamic effects of FGF2 and the 5-HT1A agonist 8-OH-DPAT on 5-HT1A receptor homodimerization in HEK cells containing FGFR1–5-HT1A heteroreceptor complexes. (A) BRET^2 acceptor saturation curves were performed by transfecting HEK293T cells with a constant DNA concentration of donor 5HT1A-Rluc8 and increasing concentrations of acceptor 5-HT1A-GFP2 constructs in presence of a constant DNA concentration of FGFR1 (wild-type). BRET^2 ratio, total fluorescence, and total luminescence as well as transformed values into receptor numbers were determined as described in Section 2 and Supplementary material Fig. 1. Cells were pre-incubated 10 min with vehicle, 8-OH-DPAT (100 nM), FGF-2 (50 ng/ml), or with both 8-OH-DPAT and FGF-2 (100 nM and 50 ng/ml). A highly significant increase in the quantitative BRET^2 signal was found for the 5-HT1A homodimer when analyzed in the presence of combined agonist treatment not seen with single agonist treatment ($P < 0.001$). The curves represent the means of saturation curves ($n = 6$) that were fitted using a non-linear regression equation assuming a single binding site. The 5-HT1A-Rluc8/5-HT1A-GFP2 curve fitted better to a saturation curve than to a linear regression, F test ($P < 0.01$). (B) After normalization the BRET50 values were significantly reduced by combined 8-OH-DPAT and FGF-2 treatment versus vehicle and 8-OH-DPAT alone ($P < 0.001$).

co-transfected at a constant ratio (1:1:1) with 5-HT1A, FGFR1-Rluc8 and FGFR1-GFP2. A mixture of cells expressing singly FGFR-Rluc8 or FGFR-GFP2 was used as a negative control. As a positive control was used cells expressing a GFP2-Rluc tandem fusion protein.

FGF2 alone at the 5 min time-point produced an increase in the BRET^2 signal above 90% of the basal value (Fig. 2A). The combined treatment with FGF2 and the 5-HT1A agonist resulted in a statistically significant further increase of the transfer of energy between FGFR-Rluc8 and FGFR-GFP2 (Fig. 2A). This enhancing effect was counteracted by the 5-HT1A antagonist S-WAY 100135 (1 μM) (Fig. 2A). Similar results were obtained with FGF20, which alone produced an increase in the BRET^2 signal which was significantly and markedly reduced compared to that obtained with FGF2 (Fig. 2A).

The kinetics of the FGFR1-Rluc/FGFR1-GFP2 interaction after FGF2 treatment and its modulation by 8-OHDPAT was also studied in transiently transfected HEK293T-27 cells using the BRET^2 assay to study the FGFR1 homodimer over a period of 10 min. As shown in Fig. 2B, FGF-2 and the combined FGF-2 and 8-OH-DPAT treatments showed no clear-cut changes of the BRET^2 value over the 10 min period. However, the combined treatment had a weak tendency to increase the BRET^2 signal over time whereas the FGF2 alone treatment had a modest tendency to decrease the BRET^2 signal over time. By contrast, treatment with FGF-20 alone resulted in a rapid and marked reduction of the BRET^2 signal from the FGFR1 homodimer during the first 10 min which was significantly different compared with FGF-2 (alone and with 8-OH-DPAT). Combined FGF-20 and 8-OH-DPAT treatment counteracted this decline in the BRET^2 signal found with FGF20 alone (Fig. 2B).

A concentration–response curve with FGF-2 was performed on the development of the BRET^2 signal from the FGFR1 homodimer in the HEK293T cells. The cells were transiently co-transfected at a constant ratio (1:1:1) of 5-HT1A, FGFR1-Rluc8 and FGFR1-GFP2 and treated with the agonist ligands for 5 min before BRET^2 measurement. Treatment with 8-OH-DPAT (with two different concentrations: 50 nM and 250 nM) shifted the curves of the BRET^2 signal to the left with the highest concentration being more effective. These results indicate an enhanced potency of combined treatment with FGF2 and the 5-HT1A agonist vs FGF-2 treatment alone to promote FGFR1 homodimer formation.

3.3. Effects of combined and single treatment with FGF2 and 8-OH-DPAT on 5-HT1A–β-arrestin2 interaction in HEK cells containing FGFR1–5-HT1A heteroreceptor complexes

β-Arrestin2 is known to mediate internalization and recycling of 5-HT1A receptors [14]. Therefore, FGF2 modulation of the 5-HT1A–β-arrestin2 interaction was studied in HEK293T-27 cells containing the 5-HT1A-FGFR1 heteroreceptor complexes using BRET^2 assays. The kinetics of the 5-HT1A-β-arrestin2 recruitment and interaction was carried out in HEK293T-27 cells transiently cotransfected with FGFR1, 5-HT1A-Rluc8 and β-arrestin2-GFP2 at a fixed ratio (1:1:2). Stimulation of 5-HT1A-Rluc8 with 250 nM 8-OH-DPAT induced a rapid rise of the BRET^2 signal, which was almost stable for 20 min (Fig. 3A). The increase in the BRET^2 signal by 8-OH-DPAT was completely counteracted when the cells were treated with the 5-HT1A antagonist S-WAY 100135 (10 uM). In addition, when cells were treated with 250 nM 8-OH-DPAT in the presence also of FGF2 (30 ng/ml) the BRET^2 signal became significantly increased versus 8-OH-DPAT treatment alone in the first 5 min period. Then it decreased reaching a stable plateau signal after 12 min not different from that reached with 8-OH-DPAT alone. It should be noted that FGF2 (30 ng/ml) incubation alone produced a small and stable rise of the BRET^2 signal over the time period analyzed (20 min) (Fig. 3A).

3.4. Effects of treatment with FGF2 on FGFR1–β-arrestin2 interaction in HEK cells

Experiments were carried out to study if FGF2 can increase the interaction between FGFR1 and β-arrestin2 in HEK cells lacking 5-HT1A receptors. BRET^2 experiments were performed in HEK293T-27 cells transiently co-transfected with a constant ratio (1:2) of FGFR1-Rluc8 and β-arrestin2-GFP2. Cells were incubated with

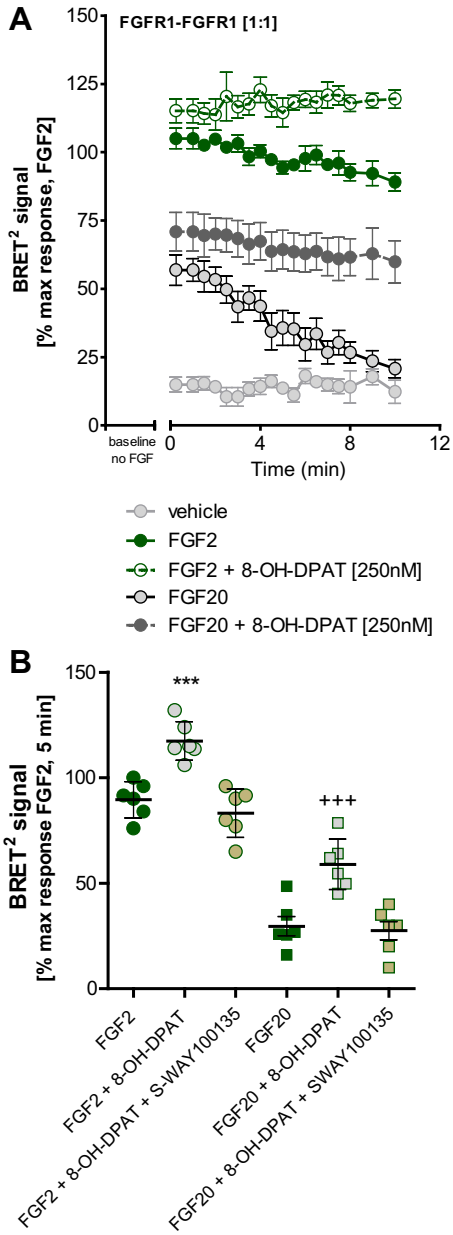

Fig. 2. Effects of combined and single treatment with 8-OH-DPAT and FGF2 on FGFR1 homodimerization in HEK293T27 cells containing FGFR1–5-HT1A heteroreceptor complexes. (A) The kinetics of the FGFR1-Rluc8/FGFR1-GFP2 interaction after FGF2 or FGF20 treatments and its modulation by 8-OHDPAT was studied over a period of 10 min in presence of heparin (0.5 μM). FGF-2 and the combined FGF-2 and 8-OH-DPAT treatments showed no clear-cut changes of the $BRET^2$ value over the 10 min period. However, the combined treatment had a weak tendency to increase the $BRET^2$ signal over time. Treatment with FGF-20 alone resulted in a rapid and marked reduction of the $BRET^2$ signal from the FGFR1 homodimer during the first 10 min which was counteracted by combined treatment with FGF20 and 8-OH-DPAT. (B) The agonist-induced rise of the FGFR1 homodimer $BRET^2$max value is shown in percent of the maximal response observed for a treatment with FGF2 alone in 5 min. Data represent the mean ± S.E.M. of 6–7 independent experiments performed each in triplicate. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison post-test. The *P* value 0.05 and lower was considered significant. ***: Significantly different compared to FGF-2 alone and combined treatment with FGF2, 8-OH-DPAT and S WAY 100135 ($P < 0.001$). +++: Significantly different compared to FGF20 alone and combined treatment with FGF20, 8-OH-DPAT and S WAY 100135 ($P < 0.001$).

increasing concentrations of FGF2 (10, 25 and 50 ng/ml) which resulted in an almost 70% increase in the $BRET^2$ signal over unstimulated cells (Fig. 3B). The FGFR1 agonist mediated increase in the $BRET^2$ signal was specific since treatment of the cells with 15 μM of the FGFR1 antagonist PD 173074 caused a marked counteraction of the FGF2 induced increase of the $BRET^2$ signal observed (Fig. 3B).

4. Discussion

The role of the allosteric receptor–receptor interactions in the FGFR1–5-HT1A heteroreceptor complexes was studied in cellular models with regard to their impact on 5-HT1AR and FGFR1 homodimerization based on $BRET^2$ assays. The findings show that coagonist treatment with 8-OH-DPAT and FGF2 but not treatment with the 5-HT1A agonist alone in FGFR1, 5HT1A-Rluc8 and 5-HT1A-GFP2 cotransfected cells significantly and markedly increases the BRETmax values and significantly reduces the BRET50 values of 5-HT1A homomers. It was previously demonstrated that this combined agonist treatment also increases the density and affinity of the FGFR1–5-HT1A heteroreceptor complexes (see [9]). The current results strongly support that the observed increases in 5-HT1A homodimers are part of the FGFR1–5-HT1A heteroreceptor complexes. The coagonist treatment produces an allosteric change in the 5-HT1A protomer which leads to increased recruitment of another 5-HT1A monomer. An increased formation of a heteroreceptor complex built up of FGFR1 and a 5-HT1A homodimer takes place.

The effects of FGF2 or FGF20 with or without the 5-HT1A agonist were also studied on the FGFR1 homodimerization using $BRET^2$ assays in HEK293T cells transiently cotransfected with 5-HT1A/FGFR1-Rluc8 and FGFR1-GFP2. FGF2 produced a marked and rapid increase in FGFR1 homodimerization which partially declined over a 10 min period. Cotreatment with the 5-HT1A agonist blocked this decline in FGFR1 homodimerization. Similar results were obtained with FGF20 which, however, was less effective in increasing the $BRET^2$ signal and thus the FGFR1 homodimerization. The results show an impact of the 5-HT1A agonist since it counteracted the dissociation of the FGFR1 homodimer. They strongly support that also the increases in FGFR1 homomers can be part of the FGFR1–5-HT1A heteroreceptor complex. Agonist coactivation results in a heteroreceptor complex where the FGFR1 homodimer contribution increases. In further support of this view the effects of the 8-OH-DPAT are blocked by a 5-HT1A receptor antagonist. Furthermore, the 5-HT1A receptor agonist concentration dependently increases the potency of FGF2 to increase the $BRET^2$ signal and thus FGFR1 homodimerization. The existence of a facilitatory allosteric 5-HT1A–FGFR1 interaction increases the formation of FGFR1 homodimers within the heteroreceptor complex.

The 5-HT1A receptors are known to be internalized and recycled via recruitment of β-arrestin2 [14]. In line with these results it was found that the 5-HT1A agonist in HEK293T-27 cells, transiently cotransfected with FGFR1, 5-HT1A-Rluc8 and β-arrestin2-GFP2, markedly increased the $BRET^2$ signal from the 5-HT1A-β-arrestin2 receptor–protein complex. The specificity was demonstrated by the observation that the 5-HT1A receptor antagonist WAY 100135 blocked the action of 8-OH-DPAT. FGF2 only produced a small increase in the $BRET^2$ signal from this complex which was additive to the effect of 8-OH-DPAT as seen from the results with combined agonist treatment. However, the additivity was mainly found during the first 10 min period. These results indicate that the allosteric FGFR1–5-HT1A receptor–receptor interaction increases the ability of the 5-HT1A receptor protomer to recruit β-arrestin2. They can in part be explained by the findings that combined agonist treatment through the receptor–receptor interaction substantially increases the formation of 5-HT1A receptor homodimers likely leading to increased β-arrestin2 recruitment. This can be associated with an increase in the internalization of the heteroreceptor complex.

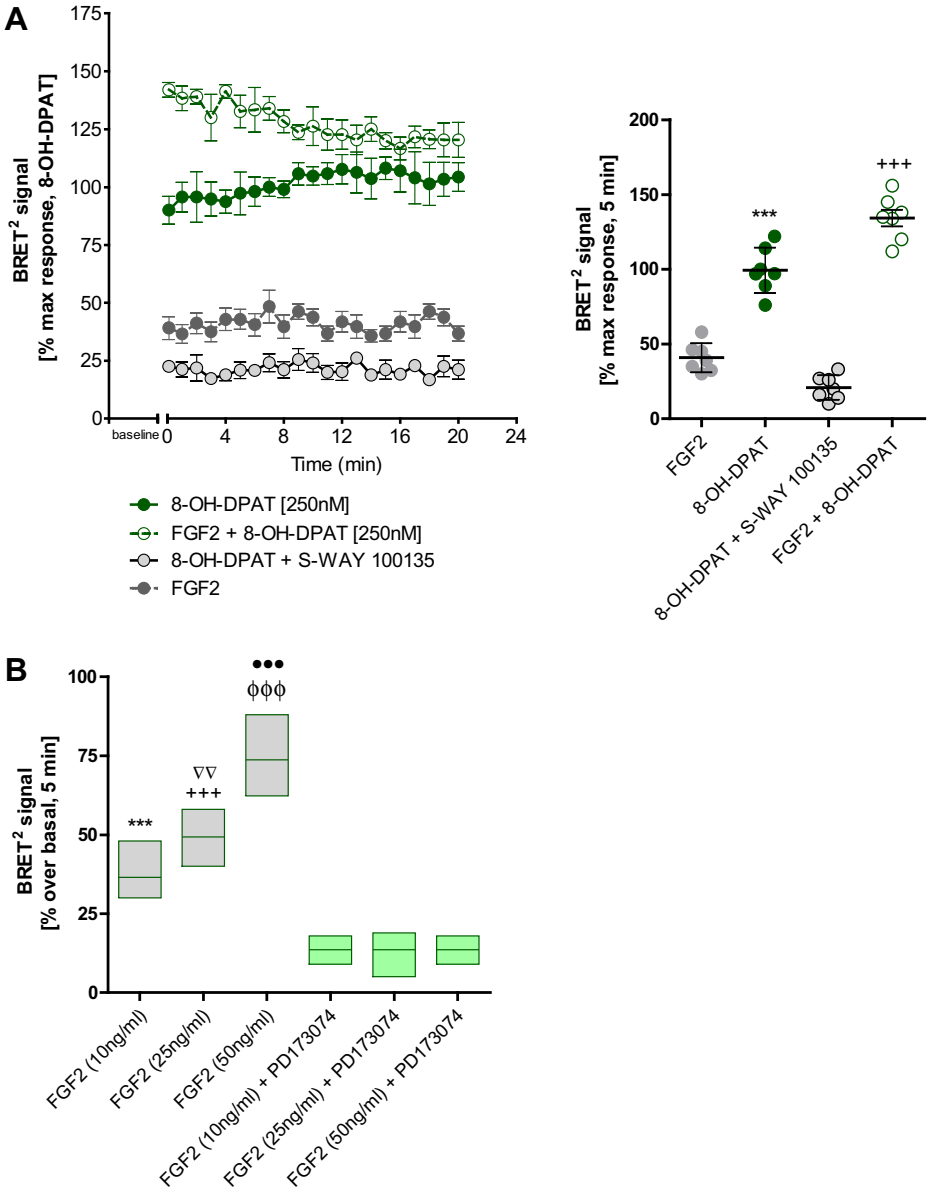

Fig. 3. BRET^2 studies of β-arrestin2 recruitment to 5-HT1A and FGFR1 protomer in HEK cells containing FGFR1–5-HT1A heteroreceptor complexes. (A, left) The kinetics BRET^2 analysis of the β-arrestin2 recruitment to 5-HT1AR was carried out over a period of 20 min. Mean ± S.E.M.; $n = 8$ in triplicate. (A, right) The agonist-induced rise of the 5-HT1A-β-arrestin2 BRET^{2}max value is shown as a percent of the maximal response to 8-OH-DPAT after 5 min incubation time. Data represent the mean ± S.E.M.; $n = 7$ in triplicate. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison post-test. The P value 0.05 and lower was considered significant. ***: Significantly different compared to FGF-2 alone and combined treatment 8-OH-DPAT and S WAY 100135 ($P < 0.001$). $^{+++}$: Significantly different compared to FGF2 alone and 8-OH-DPAT plus S-WAY 100135 ($P < 0.001$). (C) Study on the FGF2 induced β-arrestin2 recruitment to the FGFR1 protomer by means of BRET^2 analysis. HEK293T27 cells were transiently co-transfected with a constant ratio (1:2) of FGFR1-Rluc8 and β-arrestin2-GFP2 and treated with increasing concentrations of FGF2 (10, 25 and 50 ng/ml) or combined treatment of the same agonist concentrations with the FGFR1 antagonist PD 173074 (15 μM). Data represent the mean with minimum and maximum values; $n = 7$ in triplicate. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison post-test. The P value 0.05 and lower was considered significant. ***: Significantly different compared to FGF2 10 ng/ml + PD 173074 group ($P < 0.001$). +++: Significantly different compared to FGF2 25 ng/ml + PD 173074 group ($P < 0.001$). ϕϕϕ: Significantly different compared to FGF2 50 ng/ml + PD 173074 group ($P < 0.001$). ΔΔ: Significantly different compared to FGF2 10 ng/ml ($P < 0.01$) and •••: significantly different compared to FGF2 25 ng/ml ($P < 0.001$).

Also, BRET^2 experiments carried out in HEK293T-27 cells transiently co-transfected with FGFR1-Rluc8 and β-arrestin2-GFP2 demonstrated that FGF2 produced a concentration dependent increase in the BRET^2 signal due to increased formation of FGFR1–β-arrestin2 receptor protein complexes. This effect was blocked by the FGFR1 antagonist PD173074. B-arrestin2 may thus directly bind the FGFR1 homodimer upon its activation by FGF2. RTKs were previously found to recruit β-arrestin [15]. Strong support is therefore obtained that the increased formation of 5-HT1A–β-arrestin2 complexes, likely involving the 5-HT1A homodimer (see above), also can be associated with increased formation of FGFR1–β-arrestin2 receptor–protein complexes. The geometry of the 5-HT1A–FGFR1 heteroreceptor complex (Fig. 4) may allow such β-arrestin2 complexes to be formed as well.

Taken together, the results indicate a dynamic agonist regulation of the FGFR1–5-HT1A heteroreceptor complexes, which via allosteric receptor–receptor interactions leads to a structural change in this heteroreceptor complex. This change results in an expanded FGFR-5-HT1A heteroreceptor complex in which an increased presence of FGFR1 and 5-HT1A homodimers develops.

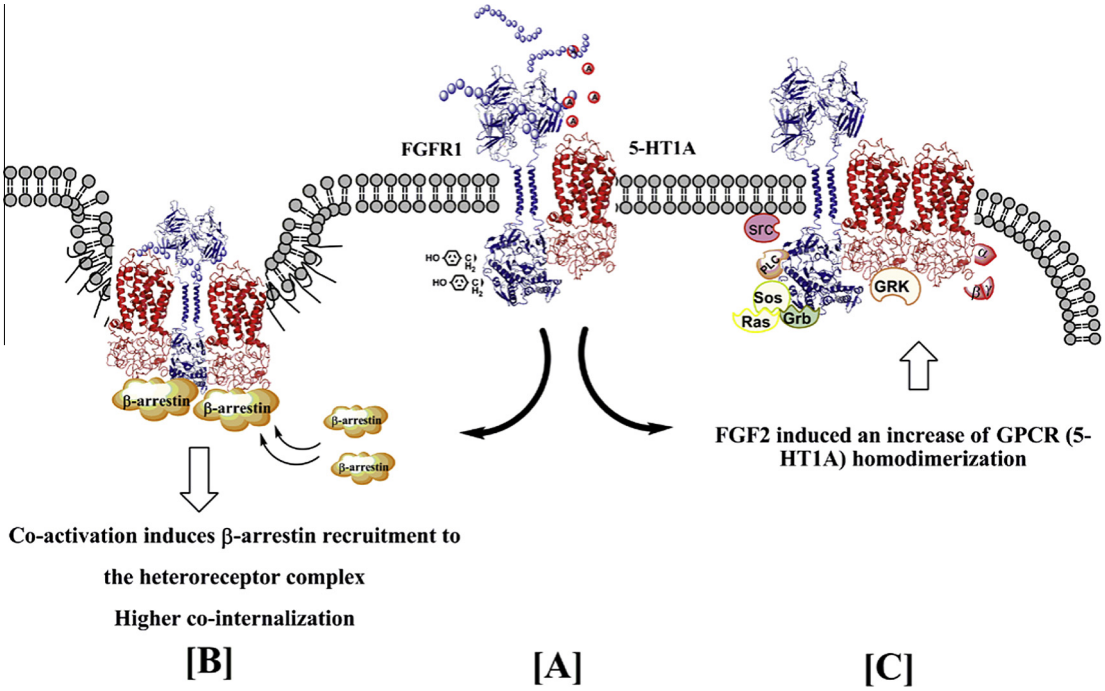

Fig. 4. Schematic illustration of the dynamic agonist regulation of the FGFR1–5-HT1A heteroreceptor complex. (A) Combined treatment with FGF2 and 5-HT1A agonists, lead to a structural change in the respective protomers which results in an expanded heteroreceptor complex as seen in B and C. (B) Recruitment of β-arrestin2 to the 5-HT1A and FGFR1 protomers upon the combined treatment with FGF2 and 5-HT1AR agonist. (C) Increased participation of 5-HT1A homodimers upon combined FGF2 and 5-HT1AR agonist treatment.

Thus, the agonist regulation of these heteroreceptor complexes produces a reorganization in their structure to include an increased participation of FGFR1 and 5-HT1A homodimers and recruitment of β-arrestin2 to the 5-HT1A and also to the FGFR1 protomers.

Acknowledgments

This work has been supported by the Swedish Medical Research Council (04X-715), Telethon TV3's La Marató Foundation 2008 and Hjärnfonden to K.F.; by grants from the Swedish Royal Academy of Sciences (Stiftelsen B. von Beskows Fond and Stiftelsen Hierta-Retzius stipendiefond) and Karolinska Institutets Forskningsstiftelser 2011 and 2012 to D.O.B.-E.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.067.

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