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β_1-Adrenergic receptor downregulates the expression of cyclooxygenase-2

Sivan Brender, Liza Barki-Harrington *

Department of Human Biology, Faculty of Natural Sciences, University of Haifa, Mt. Carmel, Haifa 3498838, Israel

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ABSTRACT

Cyclooxygenase-2 (COX-2) catalyzes the rate-limiting step in the generation of prostanoids, and is thus one of the key players in the inflammatory process. Contrary to the constitutively expressed isoform COX-1, the expression of COX-2 is rapidly and transiently upregulated following pathological stimuli but little is known about pathways that mediate its degradation. Here we show that co-expression of COX-2 together with the β_1 adrenergic receptor (β_1AR) specifically lowers the expression of COX-2 in a dose-dependent manner. We further find that stimulation of the receptor for prolonged periods of time does not reverse the β_1AR-induced decrease in COX-2, suggesting that this effect does not occur via classical β_1-mediated signaling pathways. Rather we find that the half-life of COX-2 is significantly decreased in the presence of β_1AR and that inhibition of the proteasome reverses the effect of the receptor on COX-2. Together these findings ascribe a new role for β_1AR in the downregulation of COX-2.

1. Introduction

The cyclooxygenase (COX) enzyme isoforms COX-1 and COX-2 catalyze the rate-limiting step in generation of prostanoids – bioactive lipids that play central roles in cardiovascular function. While both isoforms share a high degree of structural and catalytic similarities, differences in their expression patterns led to the formation of the COX-2 hypothesis whereby the ubiquitously expressed COX-1 is responsible for homeostatic functions, while the inducible COX-2 plays a predominant role in pathophysiological conditions (reviewed in [1,2]). Studies over several decades have underlined the necessity to inhibit the enzymatic activity of COX-2 as an efficient means of controlling inflammatory pathways. However, the prolonged use of selective COX-2 inhibitors as means of treating pathologies related to overexpressed COX-2 significantly increases the occurrence of cardiovascular events [3–5].

While the signaling cascades that lead to the induction of COX-2 are well-characterized [6], there is much less information about the regulatory pathways that mediate its degradation. In the absence of substrate, the mature COX-2 enzyme is shuttled directly from the ER via the ER-associated degradation (ERAD) pathway to the cytosol, where it is subsequently ubiquitinated and degraded by the proteasome [7–9]. This process was recently shown to be accelerated through the interaction of COX-2 with cellular proteins such as Caveolin-1 [10,11]. Recently we have demonstrated that COX-2 expression is also downregulated through its interaction with the G-protein coupled receptor (GPCR) prostaglandin E_1 receptor (EP_1) [12], suggesting that GPCRs may have a yet unrecognized function in regulating COX-2 expression. The purpose of the present study was to characterize the effect of the most prominent adrenergic receptor in the human heart, β_1 adrenergic receptor (β_1AR), on COX-2 expression and to find out if this effect is mediated via canonical signaling pathways.

2. Materials and methods

2.1. Cell culture and transfection

All transfections were done in HEK 293 cells using PolyJet (SignaGen Laboratories) as described previously [12].

2.2. cDNA constructs

oCOX-1 and hCOX-2 cDNA constructs were gift of Prof. WL Smith, University of Michigan. Flag-tagged β_1AR was gift from Prof. HA Rockman, Duke University Medical Center.

* Corresponding author. Address: Department of Human Biology, Faculty of Natural Sciences, University of Haifa, 199 Aba Khoushy Ave., Mt. Carmel, Haifa 3498838, Israel. Fax: +972 4 8288763.
E-mail address: lbarki@psy.haifa.ac.il (L. Barki-Harrington).

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2.3. Flow cytometry

Cells were washed twice with PBS, trypsinized and resuspended in 150–200 μl MEM for cytometric analysis. The samples were analyzed using BD FACSCanto II flow cytometer with DACSDiva software (BD Biosciences, San Jose, CA), as described previously [12].

2.4. Statistical analysis

All bars represent mean + SEM. Statistical analyses were done using the GraphPad Prism Software. Unless otherwise stated, statistical significance was determined by one-way ANOVA. p values < 0.05 were considered significant.

3. Results and discussion

To test whether β_1AR affects COX-2 expression, HEK293 cells were transfected with COX-2 alone or together with either empty vector or β_1AR at a 1:5 transfection ratio. As depicted in Fig. 1A, co-expression of both proteins resulted in a marked reduction in COX-2 expression. Similar results were obtained by flow cytometry using YFP-tagged COX-2 (Fig. 1B). In order to test whether the effect of β_1AR on COX-2 is dependent upon the quantity of β_1AR, COX-2 was expressed at a constant concentration with increasing amounts of β_1AR. Exact total DNA levels were kept in all samples using empty vector. As shown in Fig. 1C gradual increase in β_1AR levels led to a decrease in COX-2 levels, suggesting the effect is dose-dependent. A similar experiment performed with YFP-COX-1 showed a considerably lower effect of the receptor in comparison to COX-2.

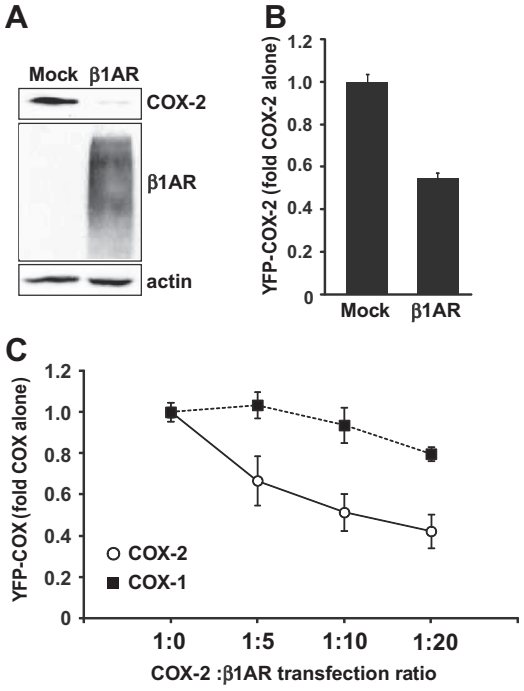

Fig. 1. β_1AR downregulates the expression of COX-2. (A) Representative Western blot of lysates obtained from cells transfected with 0.15 μg COX-2 and 0.75 μg of either Mock or β_1AR for 16 h. (B) HEK 293 cells transfected with YFP-COX-2 and either Mock or β_1AR were transfected under the same conditions as above were analyzed using flow cytometry. $N = 5$ independent experiments in triplicates. (C) Dose–response effect of β_1AR on YFP-COX-2 and YFP-COX-1 expression. Cells were transfected with different ratios of COX: receptor as indicated in the graph. Total amounts of DNA were kept the same in all samples using empty vector. $N = 4$ independent experiments in triplicates.

We next investigated whether stimulation of the β_1AR is required in order to evoke its effect on COX-2. For this, cells expressing YFP-COX-2 with either an empty vector or β_1AR were stimulated with the β_1AR-selective agonist dobutamine from 0–300 min. Cells were pretreated with the β_2AR antagonist ICI 118,551 for 30 min to block β_2ARs that are endogenously expressed in these cells. Following stimulation, cells were washed and collected 5 h later to allow for recovery of protein levels. Receptor stimulation was confirmed by measuring activation of the ERK MAP kinase pathway (Fig. 2A, bottom panels). As shown by both Western blot (Fig. 2A top panel) and flow cytometry (Fig. 2B), stimulation of the receptor did not affect the reduction of COX-2 by β_1AR, suggesting that the observed effect is not mediated through canonical signaling pathways.

To study whether β_1AR affects COX-2 stability we performed a time-course flow cytometry analysis, where we followed the kinetics of COX-2 degradation in the presence of the protein synthesis inhibitor cycloheximide (CHX). Cells were co-transfected with YFP-COX-2 and either empty vector or β_1AR overnight to allow for COX-2 expression, and treated the next day with CHX for 2–10 h. As shown in Fig. 3A, cells that were transfected with COX-2 and receptor expressed approximately half of the amount of the control. Furthermore, the calculated half life of COX-2 in the absence of the receptor was approximately 9 h, and was decreased by about three fold in the presence of the receptor, indicating that the receptor lowered COX-2 stability and accelerated its degradation. Finally, to test whether the decrease in COX-2 stability is due to its accelerated degradation via the proteasome, cells

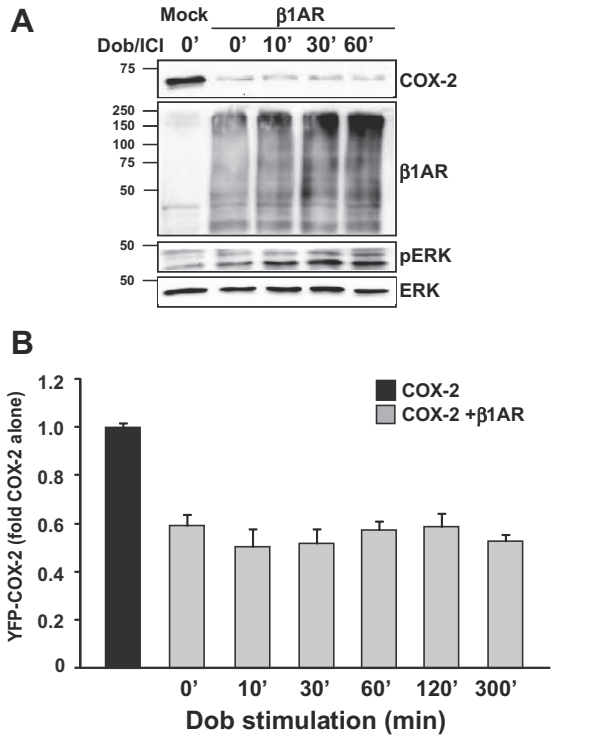

Fig. 2. The effect of β_1AR on COX-2 does not require receptor activation. (A) Representative immunoblot of HEK293 cells transfected with YFP-COX-2 and either empty vector or β_1AR and treated with 10 μM β_2AR antagonist ICI-118,551 for 30 min, prior to stimulation by 10 μM dobutamine (Dob) for the indicated time points. Cells were then washed with PBS and incubated for 5 h.YFP-COX-2 levels were assayed by Western blot using anti-COX-2 and anti-Flag antibodies. pERK and total ERK immunoblotting was performed on the same membranes to confirm activation. $N = 6$ independent experiments. (B) Cells expressing YFP-COX-2 were co-transfected with β_1AR and stimulated as above, for the indicated time points. COX-2 levels were detected by flow cytometry. $N = 7$ independent experiments in triplicates.

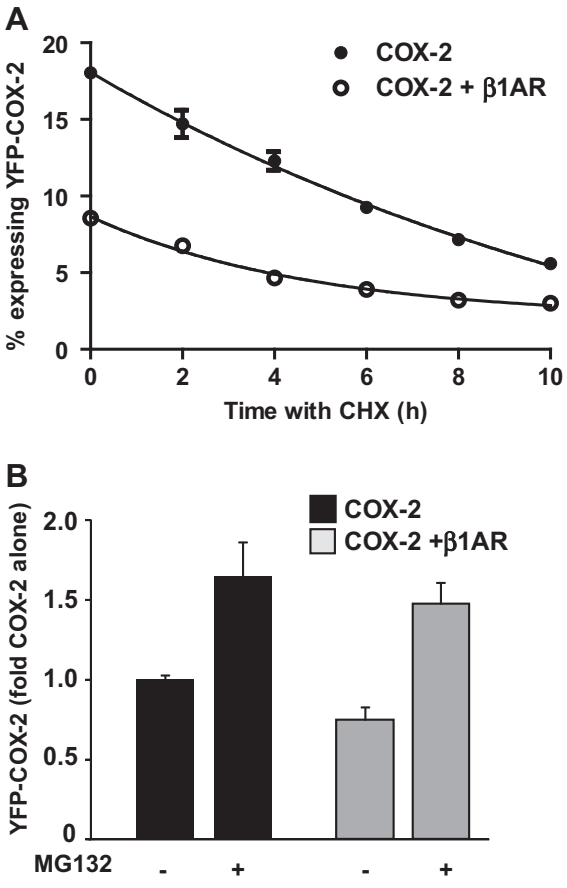

Fig. 3. β_1AR accelerates COX-2 degradation via the proteasome. (A) HEK293 cells transfected with COX-2 and either empty vector or β_1AR at a ratio of 1:5. 16 h after transfection cells were treated with 50 μM cycloheximide (CHX) for the indicated time points. YFP-COX-2 expressing cells were analyzed by flow cytometry. $N = 3$ in triplicates. (B) HEK293 cells were transfected as above and treated with or without 10 μM of MG132 for the full duration of transfection. Cells were harvested 24 h after transfection and analyzed by flow cytometry.

expressing either COX-2 alone or in the presence of β_1AR were treated with the specific proteasome inhibitor MG132 for the full duration of transfection. As displayed in Fig. 3B, inhibition of the proteasome completely abolished the reducing effect of β_1AR on COX-2.

The β_1AR is the most prominent among the adrenergic receptors that are expressed in the human heart (approximately 80% of βARs) and it mediates the positive inotropic and chronotropic responses to catecholamines through intracellular signal transduction pathways [13]. Agonist stimulation of cardiac β_1AR promotes coupling to Gs followed by a cascade of signaling that leads to activation of ERK [14]. The data presented in this study propose a new role for the β_1AR in downregulation of COX-2 expression. They also posit that the effect of β_1AR on COX-2 does not involve these pathways since it occurs in the absence of any ligand in the system, and stimulation of the receptor even for prolonged periods of time, does not change the outcome. These results are in accordance with our previous data showing downregulation of COX-2 by prostaglandin EP_1 receptor in a similar mechanism [12]. Together these studies suggest an emerging role for GPCRs beyond their traditional signaling functions, as regulators of COX-2 expression. The effect of β_1AR on COX-2 may be especially physiologically relevant since COX-2 is regularly expressed in the normal human and rodent hearts [15,16], and since chronic catecholamine stimulation following an injury to the heart causes a severe reduction in the number of and function β_1ARs [13].

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