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PTH Regulates β2-Adrenergic Receptor Expression in Osteoblast-Like MC3T3-E1 Cells

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ABSTRACT

As the aged population is soaring, prevalence of osteoporosis is increasing. However, the molecular basis underlying the regulation of bone mass is still incompletely understood. Sympathetic tone acts via beta2 adrenergic receptors in bone and regulates the mass of bone which is the target organ of parathyroid hormone (PTH). However, whether beta2 adrenergic receptor is regulated by PTH in bone cells is not known. We therefore investigated the effects of PTH on beta2 adrenergic receptor gene expression in osteoblast-like MC3T3-E1 cells. PTH treatment immediately suppressed the expression levels of beta2 adrenergic receptor mRNA. This PTH effect was dose-dependent starting as low as 1 nM. PTH action on beta2 adrenergic receptor gene expression was inhibited by a transcriptional inhibitor, DRB, but not by a protein synthesis inhibitor, cycloheximide suggesting direct transcription control. Knockdown of beta2 adrenergic receptor promoted PTH-induced expression of *c-fos*, an immediate early response gene. With respect to molecular basis for this phenomenon, knockdown of beta2 adrenergic receptor enhanced PTH-induced transcriptional activity of cyclic AMP response element-luciferase construct in osteoblasts. Knockdown of beta2 adrenergic receptors also enhanced forskolin-induced luciferase expression, revealing that adenylate cyclase activity is influenced by beta2 adrenergic receptor. As for phosphorylation of transcription factor, knockdown of beta2 adrenergic receptor enhanced PTH-induced phosphorylation of cyclic AMP response element binding protein (CREB). These data reveal that beta2 adrenergic receptor is one of the targets of PTH and acts as a suppressor of PTH action in osteoblasts. J. Cell. Biochem. 116: 142–148, 2015.

KEY WORDS: PTH; BONE; ADRENERGIC

Osteoporosis is a highly prevalent disease affecting nearly 20 million patients in the United States [Johnell and Kanis, 2006; Watts et al., 2010; Armas and Recker, 2012; Eriksen et al., 2014]. As elderly population is soaring in many countries, ageing associated diseases including cardiovascular, neuronal as well as musculoskeletal disorders are increasing and subsequently causing reduction in physical activities and eventually leading to bed-ridden conditions. In these patients, bone is lost rapidly due to the prolonged unloading or disuse status. This disuse-induced bone loss accelerates the increase in the risk of fractures that are fatal in a significant fraction of aged patients [Lyles et al., 2007]. Thus, the issue of disuse osteoporosis is critical for the QOL of patients as well as social medical cost.

Although disuse osteoporosis is observed in patients and in various animal models, underlying mechanism is still incompletely understood [Ishijima et al., 2001; Jiang et al., 2006; Barry and Kohrt, 2008; Armas and Recker, 2012]. We have observed that sympathetic tone is involved in disuse osteoporosis. Beta2 adrenergic receptor mediates sympathetic tone [Takeda et al., 2002; Elefteriou et al., 2005; Elefteriou, 2008] and has been implicated in bone loss in the animal models used to investigate pathophysiology of disuse osteoporosis [Kondo et al., 2005; Hino et al., 2006] using hind limb unloading (tail suspension). In these experiments, beta-blockers, such as propranolol or guanethidine reduce the levels of bone loss induced by hind limb unloading of mice. Beta-blocker treatment reduces unloading-induced down-regulation of bone formation as well as up-regulation of bone resorption. Thus, beta2 adrenergic receptor is acting on both sides of the two arms affecting bone mass levels. Not only pharmacological experiments but also genetic experiments show that disuse osteoporosis is less in mice deficient in dopamine beta-hydroxylase (DBH), an enzyme required for synthesis of cathecolamine that acts as

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a transmitter for sympathetic tone [Kondo et al., 2005]. Pharmacological ablation of central sympathetic neurons in ventromedial hypothalamic neuron by treating mice with gold thioglucose (GTG) also reduces the levels of disuse osteoporosis induced by unloading [Hino et al., 2006]. These observations reveal that sympathetic tone via adrenergic receptor plays a role in the pathophysiology of disuse osteoporosis at least in the mouse model of unloading.

With respect to bone cells, beta2 adrenergic receptor is expressed in both mouse and human osteoblasts as a major receptor type among the members of adrenergic receptor family [Togari et al., 1997; Takeuchi et al., 2001]. When beta2 adrenergic receptor is stimulated in wild type mice by injection with beta-agonists such as isoproterenol, bone is lost due to suppression of bone formation and enhancement of bone resorption. Both of such beta2 adrenergic receptor actions are reduced in osteopontin knockout mice [Nagao et al., 2011]. Thus, adrenergic receptor signaling in bone is modulated by molecules produced by osteoblasts such as osteopontin. Interestingly, beta2 adrenergic receptor is necessary for the anabolic actions of parathyroid hormone as its absence attenuates the anabolic effects of intermittent administration of human PTH(1–34) as observed in the knockout mice lacking beta2 adrenergic receptor [Hanyu et al., 2012]. These observations suggest the presence of a certain relation between the anabolic molecules such as PTH and beta2 adrenergic receptor. However, the regulation of beta2 adrenergic receptor expression in bone cells is still incompletely understood. PTH is an efficacious anabolic molecule for bone mass and it is currently used for the treatment of osteoporosis [Neer et al., 1999; Bouxsein et al., 2009]. However, whether beta2 adrenergic receptor expression in bone is regulated by PTH or not is not known. Therefore, we examined the effects of PTH on beta2 adrenergic receptor expression.

MATERIALS AND METHODS

CELL CULTURE

Osteoblast-like MC3T3-E1 cells were maintained in αMEM (Gibco) supplemented with 1% penicillin/streptomycin solution and 10% fetal bovine serum (FBS). These cells were cultured in the growth medium in a humidified atmosphere at 37 °C (5% CO_2 95% air) and passaged every 3–4 days.

GENE EXPRESSION ANALYSIS

For experiments, MC3T3-E1 cells were plated at 50,000/cm^2 in α-MEM supplemented with 10% FBS and 1% penicillin/streptomycin. Two days later, these cells were treated with 100 nM human PTH (1–34) for the indicated periods of time. For some experiments, MC3T3-E1 cell were plated at 10,000/cm^2 in α-MEM supplemented with 10% FBS and 1% penicillin/streptomycin. Three days later, these cells were treated with human PTH (1–34) at the indicated doses (0, 0.1 nM, 1 nM, 10 nM, 100 nM) for 1 h. Cells were lysed in Tri Reagent (Molecular research center). RNA was isolated according to the manufacturer's instructions. The reverse transcriptase reaction was performed using a High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems). Quantitative real time-RT-PCR was performed by using SYBR Green Super Mix. For the experiments to examine the mode of PTH action, culture medium was changed to α-MEM supplemented with 0.5% FBS and the cells were cultured in the presence or absence of cycloheximide (CHX, 2 μg/ml) or 5,6-dichloro-1-b d-ribofuranosylbenzimidazol (DRB, 25 μg/ml) for 1 h. Then, these cells were subsequently treated with 100 nM human PTH(1–34).

RNA INTERFERENCE ASSAY

For transfection with si-RNA, MC3T3-E1 cells were plated at 10,000 cells/cm^2 in α-MEM supplemented with 10% FBS. These cells were transfected with si-RNA for Adrb2 (Promega) or control si-RNA (Ambion) using Lipofectamine RNAiMAX (Invitrogen). Adrb2 mRNA levels in the cells were then examined based on real-time PCR at 48 h of transfection. MC3T3-E1 cells were cultured in the presence or absence of 100 nM of human PTH (1–34) for 1 h and then the cells were subjected to RNA extraction and subsequent real-time PCR analysis.

TRANSFECTION AND LUCIFERASE ASSAY

MC3T3-E1 cell were seeded in 24-well plates at 10,000 cells/cm^2 in α-MEM supplemented with 10% FBS. Next day, the cells were transfected with 0.1 μg of luciferase reporter plasmid containing cAMP response element that is linked to luciferase (CRE-Luc) and 20 ng of pGL4.74 expression vector using Lipofectamine 2000 (Invitrogen). These cells were co-transfected with si-RNA for Adrb2 or control si-RNA. The cells were cultured in the presence or absence of experimental compounds including PTH or forskolin for 6 h and then lysed in a lysis buffer (Promega). Some cells were co-tansfected with an expression vector for constitutively active mutant of PTH receptor, H223R (caPPR) for 24 h [Calvi et al., 2001]. Luciferase activities in the cell lysates were measured based on light using LIMAT LB9507 (Berthold). The data were normalized to those of pGL4.74 activity.

WESTERN BLOT ANALYSIS

MC3T3-E1 cells were plated at 10,000/cm^2 in α-MEM supplemented with 10% FBS in 6-well plates. Next day, the cells were transiently transfected with Adrb2 si-RNA or control si-RNA using si-RNA transfection reagents for 24 h. Before PTH treatment, medium was changed to α-MEM supplemented with 0.5% FBS for 3 h. The cells were then treated with 100 nM human PTH(1–34) or vehicle for 15 min. After treatment with PTH, these cells were rinsed twice with PBS and then lysed in ice-cold RIPA buffer (25 mm Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1%SDS). The lysates were subjected to centrifugation at 15,000/*g* for 15 min at 4 °C. The protein contents of the supernatants were determined using DC protein assay reagent (Bio-Rad Laboratories, Inc.). Aliquots of cell lysates containing 10 μg total protein were boiled for 5 min in 4 × SDS sample buffer, centrifuged (12,000/*g*, 1 min), and placed on ice. The proteins were electrophoresed in SDS/PAGE. The protein samples were transferred electrophoretically to a membrane for 1 h. After blocking the membrane in Tween–Tris-buffered saline containing 0.05% Tween 20, 150 mM NaCl, and 20 mM Tris-HCl, pH 8.0, and 5% (wt/vol) nonfat dry milk, membrane was probed with appropriate antibodies (diluted 1:1,000). CRE binding (CREB) protein and phosphorylated CREB were detected by anti-CREB antibody and polyclonal anti- phospho-CREB antibody (Cell Signaling Technologies), respectively. Visualization was carried out based on ECL Plus Western Blotting Detection System (GE Healthcare).

STATISTICAL ANALYSIS

Data were evaluated based on Student's *t*-test and two-way ANOVA. Data are expressed as mean ± SEM, and *P*-values less than 0.05 or 0.01were considered to be statistically significant.

RESULTS

PTH TREATMENT SUPPRESSES EXPRESSION OF ADRB2 MRNA LEVELS

As mouse calvaria-derived MC3T3-E1 cells are used as an osteoblast-like cell model, we chose these cells to examine the PTH regulation of beta2 adrenergic receptor expression. In terms of PTH treatment at 100 nM immediately down regulated beta2 adrenergic receptor mRNA expression levels at 1 h (Fig. 1). The levels of beta2 adrenergic receptor mRNA in these osteoblasts began to resume after 3 h of the treatment and then returned to original levels by 6–12 h (Fig. 1). These data indicate that beta2 adrenergic receptor mRNA expression is one of the targets of PTH treatment in these osteoblasts.

PTH TREATMENT SUPPRESSES ADRB2 mRNA EXPRESSION IN A DOSE DEPENDENT MANNER

As PTH is acting normally at low concentration and it is secreted very quickly as fast as within a few minutes from parathyroid gland when calcium is necessary upon hypocalcemic situation, we further examined at an early time point the dose response profile of the beta2 adrenergic receptor mRNA regulation by PTH in these MC3T3-E1 cells. The immediate early down-regulation of beta2 adrenergic receptor mRNA expression by PTH at 1 h was observed at concentration as low as 1 nM (Fig. 2). This down regulation was dose dependently enhanced when the PTH concentration was increased to 10 nM and 100 nM (Fig. 2). A very low PTH concentration such as 0.1 nM also tended to down-regulate beta2 adrenergic receptor mRNA expression in these osteoblasts. Thus, beta2 adrenergic receptor mRNA expression is regulated not only by PTH at super-physiological concentrations but also at the levels that are close to a physiological range.

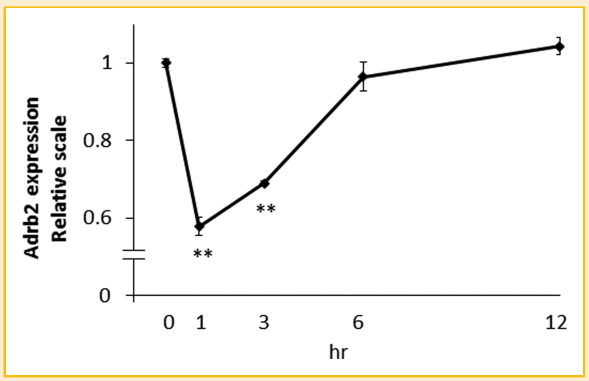

Fig. 1. PTH treatment suppresses expression of Adrb2 mRNA levels. MC3T3-E1 cells were treated with 100 nM human PTH (1–34) for indicated periods of time. At each time point, mRNA was prepared and Adrb2 mRNA levels were quantified based on real time RT-PCR. The data are normalized against those of GAPDH n = 3. Data are expressed as mean ± SEM; $^{**}P < 0.01$ compared with data at 0 h.

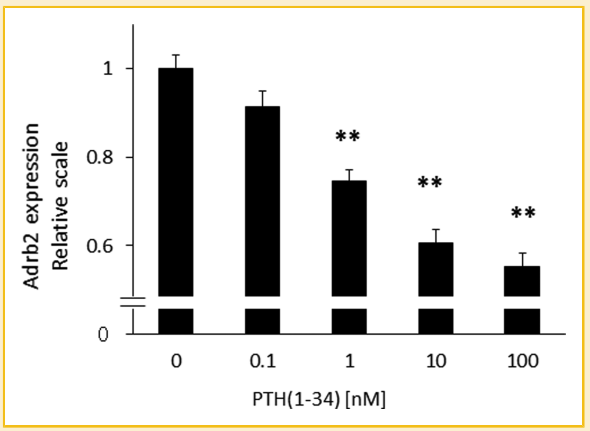

Fig. 2. PTH treatment suppresses Adrb2 mRNA expression in a dose dependent manner. MC3T3-E1 cells were treated with the indicated concentrations of human PTH (1–34) for 1 h. Adrb2 mRNA levels were quantified based on real time RT-PCR. The data are normalized against those of GAPDH n = 3. Data are expressed as mean ± SEM; $^{**}P < 0.01$.

PTH ACTIONS ON ADRB2 mRNA EXPRESSION ARE BLOCKED BY TRANSCRIPTION INHIBITOR BUT NOT BY PROTEIN SYNTHESIS INHIBITOR

We then examined the mode of PTH regulation of beta2 adrenergic receptor mRNA expression in these MC3T3-E1 cells. When transcription was inhibited by culturing these cells in the presence of a transcription inhibitor, DRB (5,6 dichloro-1-beta D-ribofuranosyl-benzimidazole), PTH down regulation of beta2 adrenergic receptor mRNA expression was no longer observed (Fig. 3). On the other hand, when MC3T3-E1 cells were cultured in the presence of a protein synthesis inhibitor, cycloheximide, PTH treatment still down-regulated beta2 adrenergic receptor mRNA expression in these cells revealing that intermediate protein synthesis is not necessary for PTH to exert its action on beta2 adrenergic receptor mRNA expression (Fig. 3). These data suggest that PTH treatment transcriptionally down-regulates beta2 adrenergic receptor mRNA expression without requiring de novo protein synthesis.

ADRB2 KNOCKDOWN ENHANCES PTH-INDUCED *C-FOS* GENE EXPRESSION

We further examined whether down regulation of beta2 adrenergic receptor mRNA expression in these osteoblasts may or may not have any functional role. To this end, we examined the effects of the down regulation of beta2 adrenergic receptor mRNA expression in these osteoblasts on PTH-induced expression of genes encoding phenotype related molecules. As PTH-induced down regulation of beta2 adrenergic receptor mRNA expression in these osteoblasts was an immediate event, we examined PTH-induced early induction of *c-fos* gene expression in osteoblasts to test this point [Pearman et al., 1996; Tyson et al., 1999; McCauley and Koh-Paige, 2001; Tanaka et al., 2004; Jilka, 2007]. For this analysis, si-RNA targeted to beta2 adrenergic receptor mRNA (si-Adrb2) or corresponding control si-RNA (si-Crtl) were used. The si-RNA constructs were transfected into these osteoblasts and si-Adrb2 this transfection reduced the levels of beta2 adrenergic receptor mRNA by about 50%

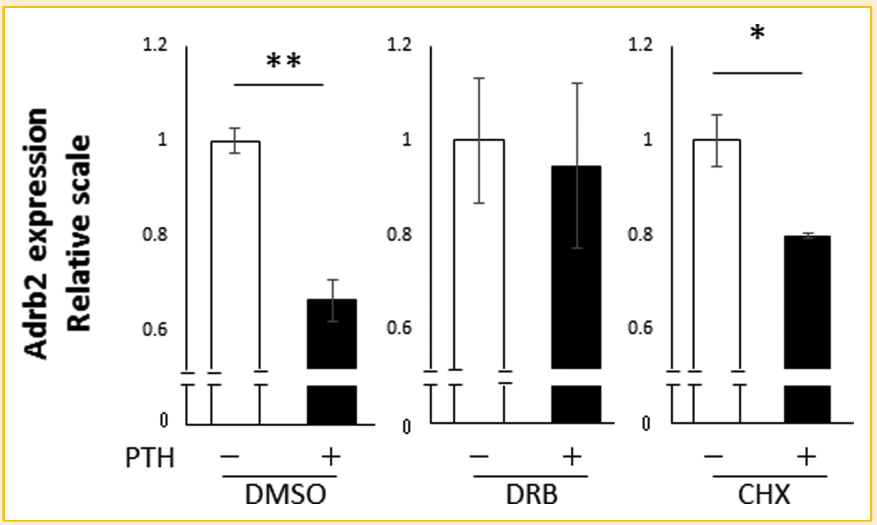

Fig. 3. PTH actions on Adrb2 mRNA expression are blocked by transcription inhibitor but not by protein synthesis inhibitor. MC3T3-E1 cells were treated with 100 nM human PTH (1–34) in the presence or the absence of 25 μg/ml DRB or 2 μg/ml cycloheximide (CHX) for 1 h. Adrb2 mRNA levels were quantified based on real time RT-PCR. The data are normalized against those of GAPDH n = 3. Data are expressed as mean ± SEM; *$P < 0.05$, **$P < 0.01$.

in the absence of PTH (i.e., control culture) while control si-RNA did not affect it. PTH induced *c-fos* gene expression about two fold as known previously. PTH-induced *c-fos* gene expression was further enhanced (about 3.5-fold) when beta2 adrenergic receptor mRNA was down regulated by si-RNA (Fig. 4). These data indicate that beta2 adrenergic receptor acts as an intrinsic inhibitory molecule for the immediate early action of PTH to induce *c-fos* gene expression in these MC3T3-E1 cells.

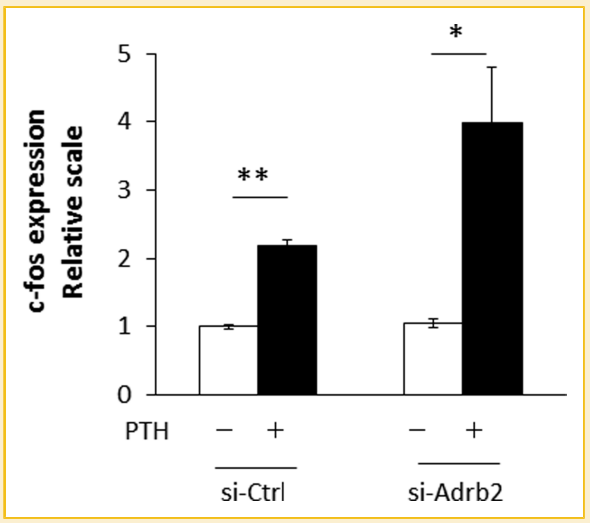

Fig. 4. Adrb2 knockdown enhances PTH-induced *c-fos* gene expression. MC3T3-E1 cells were transfected with si-control or si-Adrb2 and were cultured in the presence of 100 nM human PTH (1−34) for 1 h. Then, *c-fos* mRNA levels were quantified by real time RT-PCR. The data are normalized against those of GAPDH n = 6. Data are expressed as mean ± SEM; *$P < 0.05$, **$P < 0.01$.

ADRB2 KNOCKDOWN ENHANCES PTH-INDUCED TRANSCRIPTION OF CRE LUCIFERASE REPORTER GENE

As we observed that the action of beta2 adrenergic receptor in osteoblasts is to suppress PTH actions in these cells, next question was the levels of cellular events where beta2 adrenergic receptor regulates PTH actions. To address this point, we used a plasmid construct in which *luciferase* (*Luc*) gene was linked to cyclic AMP response element (CRE) (pCRE-Luc construct). When this reporter construct was co-transfected with control si-RNA (si-Crtl) into MC3T3-E1 cells, PTH treatment increased transcription of luciferase reporter gene expression about 80% (Fig. 5). In contrast, when the pCRE-Luc reporter construct was co-transfected with si-Adrb2, PTH-induced increase in the luciferase reporter gene expression was further up-regulated by about 250% (Fig. 5). Thus, beta2 adrenergic receptor is suppressive against PTH actions on the transcriptional events monitored by using pCRE-Luc construct.

ADRB2 KNOCKDOWN TENDS TO ENHANCE caPPR(H223R)-INDUCED TRANSCRIPTION OF CRE LUCIFERASE REPORTER GENE

Since, PTH activates its receptor to exert its effects, we examined whether beta2 adrenergic receptor regulates PTH actions by acting at the level of PTH receptor. A constitutively active mutant form of PTH receptor has been reported in human disease of Jansen type of metaphyseal dysplasia. Therefore, we used an expression vector of this mutant form of PTH receptor, having amino acid conversion from histidine at 223 to arginine (caPPR-H223R) [Schipani et al., 1997]. Co-transfection of caPPR-H223R expression vector enhanced pCRE-Luc dependent luciferase activity as known before. Knockdown of beta2 adrenergic receptor by co-transfection si-Adrb2 tended to further enhance such luciferase activity (Fig. 6).

ADRB2 KNOCKDOWN ENHANCES FORSKOLIN-INDUCED TRANSCRIPTION OF CRE LUCIFERASE REPORTER GENE

In terms of downstream events, normal PTH receptor activation by PTH leads to activation of adenylate cyclase. We therefore further

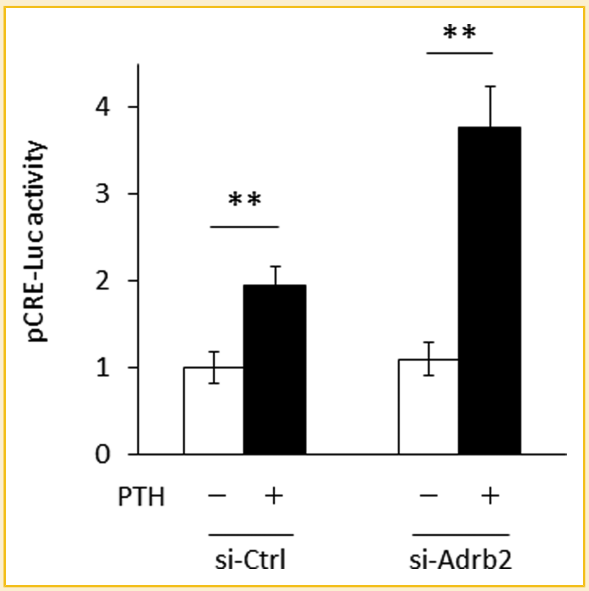

Fig. 5. Adrb2 knockdown enhances PTH-induced transcription of CRE luciferase reporter gene. CRE-Luc activity (pCRE-Luc) was examined in control cells (si-control) and cells transfected with si-Adrb2. These cells were treated with either vehicle or 100 nM PTH for 6 h. Treatment with si-RNA for Adrb2 enhanced the PTH-induced increase in the levels of luciferase activity n = 6. Data are expressed as mean ± SEM; $^{**}P < 0.01$.

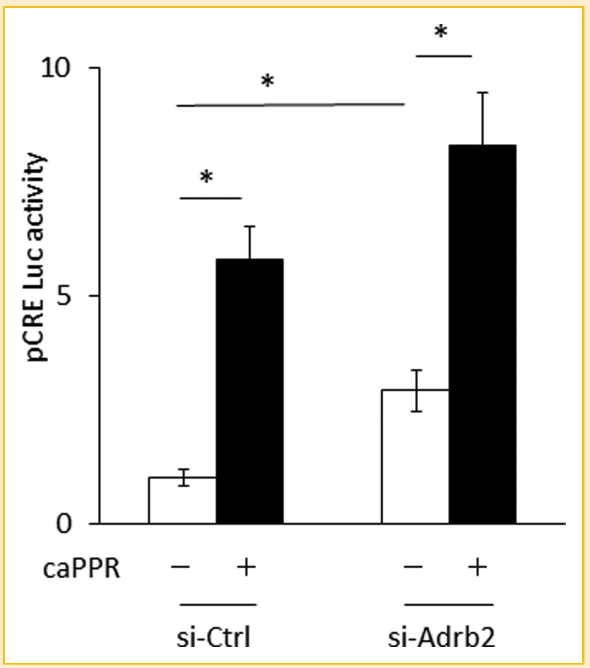

Fig. 6. Adrb2 knockdown tends to enhance caPPR (H223R)-induced transcription of CRE luciferase reporter gene. Adrb2 suppressed caPPR induced transcription. CRE-Luc activities (pCRE-Luc) in control cells (si-control) and cells transfected with si-Adrb2 in response to caPPR (H223R) were examined. caPPR (H223R) or pcDNA were co-transfected with si-control or si-Adrb2 for 24 h n = 6. Data are expressed as mean ± SEM; $^{*}P < 0.05$.

tested if beta2 adrenergic receptor may regulate PTH signaling at the level of adenylate cyclase. To do this, forskolin was used to activate adenylate cyclase directly in the presence or the absence of si-Adrb2 co-transfection. Forskolin activated pCRE-Luc activity in MC3T3-E1 cells that were co-transfected with control si-RNA (Fig. 7). Co-transfection with si-Adrb2 further enhanced such forskolin-induced luciferase activity (Fig. 7). These data suggest that beta2 adrenergic receptor suppresses PTH receptor action in osteblasts by acting at least in part at the levels of adenylate cyclase.

ADRB2 KNOCKDOWN ENHANCES PTH-INDUCED PHOSPHORYLATION OF CREB

PTH activates adenylate cyclase that leads to accumulation of cyclic AMP and subsequently activates protein kinase A (PKA). PKA phosphorylates and activates molecules including CREB, a transcription factor that binds to cyclic AMP response element to promote transcription of target genes [McCaouley and Koh-Paige, 2001; Jilka, 2007]. We therefore examined whether beta2 adrenergic receptor may regulates the PTH actions on the phosphorylation of CREB in MC3T3-E1 cells. PTH treatment enhanced phosphorylation of CREB in si-control transfected cells. Transfection of si-Adrb2 into MC3T3-E1 cells enhanced basal levels of phosphorylation of CREB and further enhanced PTH-induced phosphorylation of CREB (Fig. 8). Thus, beta2 adrenergic receptor suppresses PTH action at the levels of CREB phosphorylation.

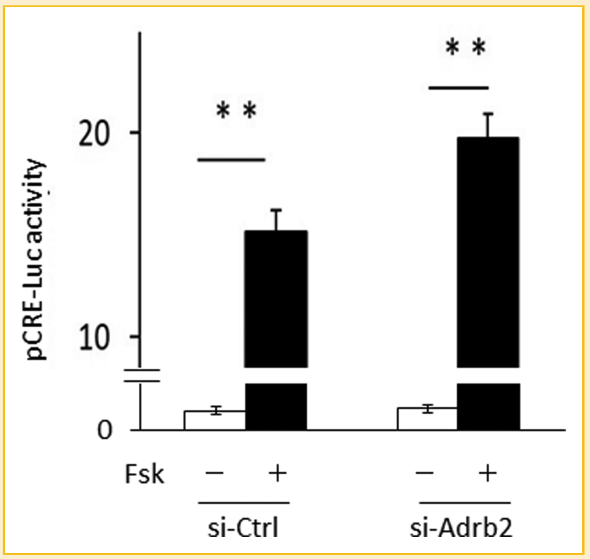

Fig. 7. Adrb2 knockdown enhances Forskolin-induced transcription of CRE luciferase reporter gene. CRE-Luc activity (pCRE-Luc) in cells transfected with control construct (si-control) and cells transfected with si-Adrb2 were examined for their response to 10 μM Forskolin (Fsk). The cells were treated with forskolin for 6 h n = 6. Data are expressed as mean ± SEM; $^{**}P < 0.01$.

DISCUSSION

PTH is a potent anabolic agent for bone while its mechanism of action has been incompletely understood. We found that PTH

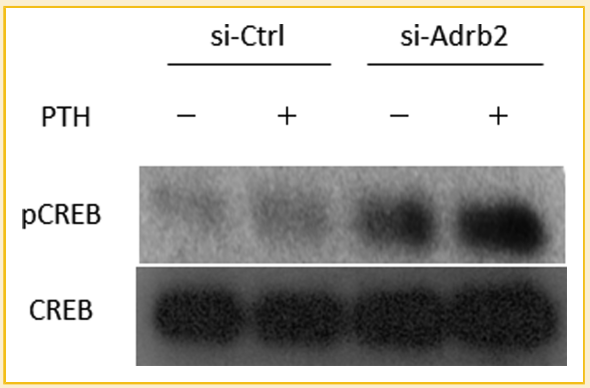

Fig. 8. Adrb2 knockdown enhances PTH-induced phosphorylation of CREB. MC3T3-E1 cells were used as described in materials and methods. Western blot analyses were conducted to examine the effects of Adrb2 knockdown on PTH-induced CREB phosphorylation. The cells were treated with 100 nM PTH for 15 min in the presence of si-negative control or si-Adrb2 and protein extracts were subjected to western blotting analyses.

suppresses the levels of beta2 adrenergic receptor gene expression in MC3T3-E1 cells, a model of the cells in osteoblastic lineage. The effects of beta2 adrenergic receptor expression in MC3T3-E1 cells is dose dependent and is observed as low as 1 nM. PTH regulation of beta2 adrenergic receptor expression is at least in part via transcriptional events and does not require new protein synthesis. As functional aspects, knockdown of beta2 adrenergic receptor mRNA by si-Adrb2 enhances PTH-induced increase in pCRE-Luc activity and enhances the effects of forskolin that is a direct activator of adenylate cyclase. Finally, transfection with si-Adrb2 enhances PTH-induced phosphorylation of CREB. These observations indicate the presence of PTH regulation of beta2 adrenergic receptor expression and suppressive function of beta2 adrenergic receptor on PTH action.

PTH receptor and beta2 adrenergic receptor are both GPCRs and are expressed in osteoblasts. However, the interaction between the two receptors is still not fully understood. In contrast to the distinct identity of the two receptor types and their respective ligands, at least some molecules that are involved in the intracellular signaling of the two receptor appear to be common. These include Gs-alpha and other G proteins, adenylate cyclase, GRK, protein kinase A, cyclic AMP, CREB, and arrestins [Feinstein et al., 2011].Though some of these molecules are shared by the two types of receptors, it appears that full pictures of the participating molecules and their roles in the events for the unique pathways as well as interacting pathways are still largely unknown. Once the nature or the repertoires and interaction of these signaling players would be identified, next step of investigation targets for the understanding of our discovery regarding the interaction between PTH receptor and beta2 adrenergic receptor may include dynamic modulation of the interactive pathways as well as identification of possible unique compartmentalization for the independent signaling events.

Intriguing properties of GPCRs are their ways of desensitization linked to transportation inside the cells. After the activation of these receptors, they are incorporated into cells and some of them are transported to endosomes in association with G-proteins and adenylate cyclase [Vilardaga et al., 2002; Wehbi et al., 2013]. These intracellular complexes are sometimes contributing to cyclic AMP accumulation while they are within the endosomes. Therefore, expression levels as well as the duration of the presence of active receptor complex inside the cells would affect the actions of GPCRs. Endosome related activity of GPCR may be different between beta2 adrenergic receptor and PTH receptor. For instance, beta2 adrenergic receptor may not seem to be incorporated into endosomes and the duration of cyclic AMP accumulation may be shorter compared to PTH. Such intracellular events may be one of the target points where beta2 adrenergic receptor and PTH receptor would interact. Our observation on the immediate early regulation of beta2 adrenergic receptor expression by PTH treatment may also be related to such short duration of the activity beta2 adrenergic receptor.

In summary, we found that PTH regulates the expression of mRNA for beta2 adrenergic receptor at least in part via transcriptional events and that beta2 adrenergic receptor that is negatively modulating PTH action.

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