FEBS Letters 589 (2015) 766–772

journal homepage: www.FEBSLetters.org

Human EP2 prostanoid receptors exhibit more constraints to mutations than human DP prostanoid receptors

Jun Tanimoto [a], Hiromichi Fujino [a,*], Hiroki Takahashi [b,*], Toshihiko Murayama [a]

[a] Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan
[b] Medical Mycology Research Center, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8673, Japan

ARTICLE INFO

Article history:
Received 1 December 2014
Revised 3 February 2015
Accepted 4 February 2015
Available online 12 February 2015

Edited by Takashi Gojobori

Keywords:
DP receptor
EP2 receptor
Prostaglandin D_2
Prostaglandin E_2
Prostanoid receptor
Tandem duplication

ABSTRACT

Human D-type prostanoid (DP) and E-type prostanoid 2 (EP2) receptors are regarded as the most closely related receptors among prostanoid receptors. Although these receptors are generated by tandem duplication, their physiological outputs often oppose one another. In the present study, we extracted mutations occurring in the coding regions of both receptors using the 1000 genome project database and found that EP2 receptors have 8-fold fewer amino acid mutations. We speculate that EP2 receptors exhibit more constraints to mutations than DP receptors.

1. Introduction

Prostanoids are lipid mediators and play very important roles in the generation of inflammatory responses. Although, inflammation is essentially beneficial for infections and injuries, it has occasionally been implicated in the pathogeneses of cancer, arthritis, stroke, and/or cardiovascular as well as neurodegenerative diseases [1]. Prostaglandin E_2 (PGE_2) is the most abundant of the five main prostanoids, throughout the body and acts as a very potent mediator that exhibits many biological activities [1,2]. The most important features of PGE_2 are the roles it plays in vasodilation, embryo implantation, contraction as well as relaxation of smooth muscle cells, gastrointestinal motility, neuroprotective functions, and inflammation [2,3]. Prostaglandin D_2 (PGD_2), a positional isomer of PGE_2, has been characterized as a regulator of sleep, and is involved in the initiation of type-I acute allergic responses including asthma in peripheral [1].

Although the diverse actions of prostanoids, including PGD_2 and PGE_2, have been characterized, these functions are inefficient if their cognate receptors, *i.e.* the prostanoid receptors, are absent. The eight types and subtypes of prostanoid receptors are the family of G-protein coupled receptors and are known as DP for PGD_2, EP1 to EP4 for PGE_2, FP for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), IP for prostaglandin I_2 (PGI_2) or prostacyclin, and TP for thromboxane A_2 (TXA_2) [4,5]. In addition to the eight prostanoid receptors, a distinct type of PGD_2 receptor belongs to the family of chemokine receptors, namely the chemo-attractant receptor-homologous molecule expressed on T helper type 2 cells (CRTH2) receptor [4,5]. Therefore, PGD_2 principally elicits its functions through the activation of DP receptors and CRTH2 receptors, whereas PGE_2 largely exerts its actions via EP1 to EP4 receptors.

The structures of PGD_2 and PGE_2 as positional isomers are very similar; however, these isomers sometimes exhibit opposite effects on the body [6]; for example, PGE_2 has been shown to induce elevations in body temperature, whereas PGD_2 lowers it [6]. PGE_2 has also been reported to suppress food intake, whereas PGD_2 increases it [6]. PGD_2 and PGE_2 exert opposite effects in the regulation of asthma, as well as the regulation of wakefulness/sleep in central.

Abbreviations: DP, D-type prostanoid; EP, E-type prostanoid; PGE_2, prostaglandin E_2; PGD_2, prostaglandin D_2; FP, F-type prostanoid; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; IP, I-type prostanoid; PGI_2, prostaglandin I_2; TP, T-type prostanoid; TXA_2, thromboxane A_2; CRTH2, chemo-attractant receptor-homologous molecule expressed on T helper type 2 cells; SNPs, single nucleotide polymorphisms; CDS, coding sequence; NCBI, National Center for Biotechnology Information

* Corresponding authors. Fax: +81 43 226 2875 (H. Fujino).
E-mail addresses: fujino@chiba-u.jp (H. Fujino), hiroki.takahashi@chiba-u.jp (H. Takahashi).

http://dx.doi.org/10.1016/j.febslet.2015.02.006
0014-5793/

The pathophysiological features of asthma are characterized by bronchial hyper-responsiveness, airway inflammation, and elevations in the levels of immunoglobulin E, which activates mast cells to produce PGD_2 [7,8]. Thus, increases in PGD_2 have been detected in asthmatic patients following an endo-bronchial allergen challenge [7,8]. Certain key regulators of asthma appear to be elicited by PGD_2 acting on DP receptors, and this has also been confirmed in studies using knockout mice [8,9]. While PGD_2 is considered to be crucially involved in the deterioration of asthma, PGE_2 may have potentially beneficial effects on this disease [10]. Thus, the activity of human and murine mast cells was found to be prevented by PGE_2 through the activation of EP2 receptors in vitro [10]. Furthermore, the activity of airway mast cells in mouse models of asthma was inhibited by both endogenous PGE_2 and/or exogenously administered PGE_2 in vivo, possibly through EP2 receptor-mediated mechanisms, which has also been supported by studies using knockout mice [10,11].

The most abundant prostanoid in the brain is PGD_2, which has been identified as a potent humoral sleep-inducing factor [12]. Previous knockout studies demonstrated that, PGD_2-activated DP receptors were important for inducing sleep, and the mechanism responsible was proposed as the production and release of adenosine [12,13]. On the other hand, the onset and maintenance of wakefulness is assumed to be regulated by PGE_2 [14,15]. PGE_2-mediated wakefulness is generally associated with the activation of EP4 receptors [15]; however, it is also reported to be mediated by EP1 and EP2 receptors [16].

In spite of the opposing effects of PGD_2/DP receptors and PGE_2/EP2 receptors on the regulation of pathological conditions such as asthma, human EP2 and DP receptors are Gαs-coupled receptors and the most homologically related receptors among prostanoid receptors [4,17–19]. Both receptor genes are located at chromosome 14q22 in humans (approximately 52.73–52.74 Mb in the DP receptor and 52.78–52.80 Mb in the EP2 receptor) and consist of very similar amounts of amino acids; 359 amino acids for DP receptors and 358 amino acids for EP2 receptors [7,20–22]. These genes are considered to be generated by tandem duplication [21]; therefore, both receptors are typically placed side by side in the phylogenetic tree of prostanoid receptors [4,19].

In the present study, we focused on extracted mutations that occurred in the coding regions of human DP and EP2 receptors using the 1000 genome project database and found that EP2 receptors had less mutations than DP receptors, especially at the amino acid level. These results suggest that constrained EP2 receptors may have irreplaceable functions, and/or DP receptors may undergo more mutations than EP2 receptors, thereby gaining new functions as a new copy of a duplicated gene.

2. Materials and methods

2.1. Identification of single nucleotide polymorphisms (SNPs) in DP and EP2 receptors

SNP data from the 1000 genomes project for *Homo sapiens* (GRCh37.p13) were used in the present study [23–26]. VCF files were processed by VCFtools (v0.1.11) [27] to obtain SNP information for each 13 of the genes examined, including DP and EP2 receptors, based on their locations on the genome, as shown in Supplementary Table I. Data for *Mus muscus* (GRCm.38.p2) were extracted from the Wellcome Trust Sanger Institute mouse genome project [28] (http://www.sanger.ac.uk/resources/mouse/genomes/), and the genome sequences of 17 key strains were processed in the same manner. SNP information was generated based on the locations of the genes in Supplementary Table I.

2.2. R/S ratio of stochastic frequency

The number of substitutions per silent site (S) and the number of substitutions per replacement site (R) of the prostanoid receptors were extracted, followed by a calculation of the R/S ratios in a similar manner to the stochastic rate, as reported previously [29].

3. Results

One of the most prevalent and important phenomena for duplicating genes is the acquisition of new functions to adapt to and/or deal with a new environment and/or condition [30,31]. However, since evolutionally complete functional redundancy is considered to be unstable in the long term, the equality of duplicated gene copies must be disrupted, *e.g.* by mutations, if they are to be preserved [32]. Therefore, to examine differences between a pair of duplicates, DP and EP2 receptors, we extracted the numbers of mutations occurring in the coding sequence (CDS) regions of human DP and EP2 receptors, using the 1000 genome project database; an integrated map from 1092 human genomes of 14 populations extracted from Europe, East Asia, sub-Saharan Africa, and the Americans [23]. As shown in Figs. 1 and 2, a total of 20 and 12 nucleotide changes were detected in the DP and EP2 receptors, respectively. Four out of the 20 nucleotides changes in DP receptors were silent mutations; therefore, their amino acid residues were conserved (Fig. 1). However, 9 out of the 12 nucleotides were silent in EP2 receptors (Fig. 2). Thus, the actual substitution of amino acids was 16 in DP receptors, but only 4 in EP2 receptors, as shown in Figs. 1 and 2. Seventeen nucleotide changes were detected and 11 amino acids were replaced with other amino acids in other Gαs-coupled human EP4 receptors; and 22 nucleotides were changed and 8 amino acids were substituted in human IP receptors (data not shown). Therefore, in terms of amino acid replacements, among Gαs-coupled prostanoid receptors, less substitutions were detected in EP2 receptors.

According to the assumption that silent mutations are largely unaffected by natural selection; therefore, the stochastic rate of the accumulation of silent mutations will be proportional to time [29]. Thus, the number of silent mutations in a pair of duplicates, in this case DP and EP2 receptors, should be similar if selective constraints do not exist; *i.e.* the evolutional stochastic rates of silent mutations will be the same. As shown in Figs. 1 and 2, the number of silent mutations was mapped by comparing the reference sequences of each receptor; NM_000953.2 for DP receptors and NM_000956.3 for EP2 receptors, respectively, and not by comparing between DP and EP2 receptors. Therefore, the numbers of mutations shown in Figs. 1 and 2 were extracted from comparisons of a pair of DP receptor reference sequences (ref_DP) and the 1000 genome project database DP receptor sequences (dbs_DP), and the same was performed for EP2 receptors. Thus, similar to the stochastic rate in evolution, the stochastic frequency of silent mutations extracted from 1092 people in the genome project in the current era should be similar between ref_DP/dbs_DP and ref_EP2/dbs_EP2 if selective constraints do not exist.

The traditional approach was useful to deduce the magnitude of selective constraint on protein evolution of comparing the rates of nucleotide substitution at replacement and silent sites [29]. Thus, the ratio of the number of substitutions per amino acid replacement site (R)/number of substitutions per silent site (S) was <1 when selective constraints existed, whereas the R/S ratio was >1, with the acceleration of protein evolution [29]. Again in this case, it is not the stochastic rate, but the stochastic frequency of 1092 people worldwide (2184 sets of genes) in the current era, the number of substitutions per silent site (S) and the number of