

Title	Roles of the Orphan Receptor Gpr176-mediated G-protein Signaling in the Central Circadian Clock(Abstract_要旨)
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論文題目	Roles of the Orphan Receptor Gpr176-mediated G-protein Signaling in the Central Circadian Clock (概日時計中枢におけるオーファン受容体Gpr176を介したG蛋白質シグナルの役割)		
<p>(論文内容の要旨)</p> <p>In mammals, the principal circadian pacemaker governing daily rhythms in behavior and physiology resides in the suprachiasmatic nucleus (SCN) of the hypothalamus. Malfunction of the circadian clock has been linked to the pathogenesis of a wide variety of diseases, including sleep-wake disorder, tumorigenesis, obesity, diabetes, and hypertension. Drug efficacy and toxicity are also under circadian regulation. These lines of evidence support the potential value of developing drugs that target the circadian clock.</p> <p>G-protein-coupled receptors (GPCRs) constitute the largest family of cell surface receptors, participating in a broad range of physiological functions. It has been appreciated that GPCRs are the most common target of pharmaceutical drugs: more than 30% of clinically marketed drugs target GPCR function. Intriguingly, there are still more than 140 orphan GPCRs whose cognate ligands are not known, and deciphering their physiological function remains a priority for both clinical and fundamental research.</p> <p>Heterotrimeric G-proteins ($G\alpha\beta\gamma$) are key components in the transmission of signals from GPCRs to downstream effector molecules. $G\alpha$ subunits cycle between active GTP-bound and inactive GDP-bound states, and the duration of activation is determined by the balance of GDP/GTP exchange (activation) and GTP hydrolysis (deactivation). The activation limb of the cycle is conducted by GPCRs; the deactivation limb is accelerated by GTPase-activating proteins (GAPs).</p> <p>Depending on the $G\alpha$ subtypes, a variety of downstream signaling pathways can be regulated. $G\alpha_z$ is a member of the $G\alpha_i$ family, which inhibits adenylyl cyclases and thereby reduces cAMP production. However, $G\alpha_z$ possesses many properties that distinguish it from the other $G\alpha_i$ family members. Firstly, differently from $G\alpha_i$, $G\alpha_z$ is mainly expressed in the brain plus platelets. Secondly, unlike $G\alpha_i$, pertussis toxin (PTX) does not inhibit $G\alpha_z$; $G\alpha_z$ lacks a cysteine residue in the fourth position from C-terminus that serves as a site for PTX-mediated ADP-ribosylation conserved in the other $G\alpha_i$ family members. Lastly, compared to $G\alpha_i$ and other $G\alpha$ subtypes, $G\alpha_z$ is unique in that it exhibits an extremely low intrinsic GTPase activity. However, physiological meaning of these unique biochemical features of $G\alpha_z$ has remained elusive.</p> <p>Regulator of G-protein signaling (RGS) proteins are GAPs for $G\alpha$ subunits. There are 20 RGS proteins, which have been shown to exhibit selectivity toward $G\alpha$ subunits. RGS16 is known to act on $G\alpha_i$ but not $G\alpha_s$. It has been reported that RGS16 is expressed in the SCN and that loss of RGS16 causes dysregulation in circadian signaling in the SCN and longer circadian behavioral activity in mice. Moreover, three independent genome-wide association studies (GWAS) demonstrated that human genetic variants of <i>RGS16</i> are associated with being a morning person. However, precise functioning of RGS16 within the SCN is poorly understood.</p> <p>In Chapter 1, I identify Gpr176 as an SCN-enriched orphan GPCR that sets the pace of circadian behavior. I also show that at the molecular level Gpr176 couples to G_z to reduce</p>			

cAMP synthesis. In **Chapter 2**, I show that RGS16 has the potential to serve as a GAP for Gz.

Chapter 1: Gpr176 is a Gz-linked orphan G-protein-coupled receptor that sets the pace of circadian behavior

I launch the SCN orphan GPCR project to (i) search for murine orphan GPCRs with enriched expression in the SCN, (ii) generate mutant animals deficient in candidate GPCRs, and (iii) analyze their roles in keeping circadian rhythms. I thereby identify Gpr176 as an SCN-enriched orphan GPCR that sets the pace of circadian behavior. Gpr176 is expressed in a circadian fashion in SCN neurons, and molecular characterization revealed that it represses cAMP signaling in an agonist-independent manner. Gpr176 acts independently of, and in parallel to, the Vipr2 GPCR, not through the canonical Gi, but via the unique G-protein subclass Gz.

Chapter 2: RGS16 serves as a GTPase-activating protein for G α_z

The mouse SCN does not show any detectable expression of known Gz-selective GAPs, RGSZ1 and RGSZ2, raising the possibility that yet unappreciated RGS protein(s) might act on Gz in the SCN. RGS16 is known to act on G α_i but not G α_s . To test the possibility that RGS16 possesses GAP activity for G α_z , *in vitro* GTPase assay is performed with purified G α_z proteins. RGS16 accelerates GTP hydrolysis of G α_z . My data support the hypothesis that RGS16 is involved in the termination of Gpr176-Gz-cAMP signaling in the SCN.

(論文審査の結果の要旨)

G蛋白質共役型受容体 (GPCR) は創薬上最も重要かつ成功確率の高いターゲットであるが、いまだにそのリガンド・機能未知のオーファン受容体が多く残されている。このような中、申請者は、本論文において、体内時計の時刻を調節するオーファン受容体Gpr176を同定し、さらにはその下流のG蛋白質シグナルの活性制御の実体を明らかにした。すなわち、第一章では、体内時計の最高位中枢である視交叉上核 (suprachiasmatic nucleus; SCN) に発現するオーファンGPCR をDNAマイクロアレイを用いて精査し、候補となる複数の遺伝子群についてノックアウトマウスを作出して表現型を探った結果、SCNに特異的に強く発現し、遺伝子欠損によってマウス個体の活動リズムの周期が短縮するオーファン受容体Gpr176を同定した。さらに申請者は本受容体の性状分析を進め、Gpr176がアゴニスト非存在下においても恒常的にcAMP産生を抑制する活性をもつGPCRであることを証明した。GPCRが細胞内のcAMP濃度を低下させる場合には抑制性G蛋白質Giを介することが一般的である。しかし興味深いことに、Gpr176は通常とは異なり、百日咳毒素非感受性のcAMP抑制性G蛋白質サブタイプGzとカップルすることを申請者は複数の生化学的および薬理学的手法を用いて明らかにした。またさらに、第二章ではこのGzシグナルの寿命制御を司るグアノシン三リン酸加水分解活性化因子としてRegulator of G protein signaling 16 (RGS16) の役割を生化学的に明らかにした。このように、本論文は、生体リズムの最高位中枢として機能するSCNニューロンにおいてリズム調整能を有するオーファン受容体Gpr176を見出し、その下流のG蛋白質シグナル制御の実体を明らかにした。生体リズムの異常を伴う不眠症や生活習慣病の根本的な是正を目指した新しいタイプの治療薬の開発につながる知見と期待される。よって、本論文は博士 (薬科学) の学位論文として価値あるものと認める。また、平成31年2月22日、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。なお、本論文は、京都大学学位規程第14条第2項に該当するものと判断し、公表に際しては、当分の間当該論文の全文に代えてその内容を要約したものとすることを認める。

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