（ 続紙 1 ）

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論文題目	Regulatory Mechanisms of Adrenal Gland Zona Glomerulosa-Specific 3β-HSD （副腎アルドステロン産生細胞特異的3β-HSDアイソフォームの発現制御機構）		

（論文内容の要旨）

The enzyme 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (3β-HSD) is essential for the biosynthesis of all active steroid hormones, including those secreted from the adrenal gland. Whereas two distinct 3β-HSD isoforms (type I 3β-HSD, which is encode by *HSD3B1*, and type II 3β-HSD, which is encoded *HSD3B2*) exist in humans, it has long been thought that type II 3β-HSD was the only isoform expressed in the human adrenal glands. However, this canonical view was recently revised due to the observation that the alternative isoform, HSD3B1, is expressed within zona glomerulosa (ZG) cells (*1*), where aldosterone is produced. Interestingly, the mouse also has two isoforms in the adrenal: one (Hsd3b1) is ubiquitous in the cortex, but the other (Hsd3b6) is ZG specific (*2*). Thus, in both species, the adrenals possess a ZG-specific isoform, in addition to the ubiquitous one. However, it remains unknown why these different enzymes are expressed simultaneously in ZG cells. Since these isozymes catalyze the same enzymatic reaction, the question remains open why the newly identified ZG-specific isoform needs to be present in the ZG cells (*3*).

Because the ZG cells are the principal place where aldosterone is produced and *in vivo* aldosterone production is under multiple dynamic regulations by angiotensin II (AngII), potassium (K^+), and circadian clock, this thesis is designed to investigate specific regulatory modes of this enzyme. The data shown for AngII (**chapter 1**), K^+ (**chapter 2**), and circadian clock (**chapter 3**) illustrate the unique responsive properties of this gene.

Chapter 1: Angiotensin II triggers expression of the adrenal gland zona glomerulosa-specific 3β-HSD isoenzyme through *de novo* protein synthesis of the orphan nuclear receptors NGFIB and NURR1

Firstly, I show that in both human and mouse, the ZG-specific 3β-HSD isoform gene expression is increased rapidly upon AngII stimulation. Then, the underlying molecular mechanisms are explored by using the human adrenocortical H295R cells. I show that the ZG isoform *HSD3B1* differs from *HSD3B2* in the ability to respond to AngII. Mechanistically, the induction of *HSD3B1* involves *de novo* protein synthesis of the nuclear orphan receptors NGFIB and NURR1. The *HSD3B1* promoter contains a functional NGFIB/NURR1-responsive element (NBRE) to which these proteins bind in response to AngII. Knockdown of these proteins and overexpression of a dominant negative NGFIB both reduce the AngII responsiveness of *HSD3B1*. Thus, the AngII-NGFIB/NURR1 pathway controls *HSD3B1*. This work reveals *HSD3B1* as a new regulatory target of AngII (*4*).

Chapter 2: Differential regulation of the type I 3β-hydroxysteroid dehydrogenase gene expression by angiotensin II and potassium in human adrenocortical H295R cells

Next, I find that *HSD3B1* does not increase in response to K^+, despite showing a drastic increase in response to AngII. In contrast, the aldosterone synthase (CYP11B2) gene expression is responsive to both AngII and K^+. Promoter analyses reveal that although both AngII and K^+ activate transcription form the Ca^{2+}/cAMP-responsive element (CRE) located in the *CYP11B2* promoter, the NBRE in the *HSD3B1* promoter that is an essential *cis*-element for the AngII-responsiveness of this gene does not provide any K^+ reactivity. Thus, the reactivity of the NBRE is confined to AngII, but that of the CRE is common for AngII and K^+. Consistent with this, AngII treatment increases expression of the NGFIB family proteins, but K^+ does not. In contrast, both AngII and K^+ increase phosphorylation of the CREB/ATF family CRE-binding proteins. Chromatin immunoprecipitation assays confirm that NGFIB protein occupies the *HSD3B1* promoter only after AngII treatment, while phospho-CREB/ATF protein binds to the *CYP11B2* promoter regardless of the type of stimuli. Thus, regulatory modes of *HSD3B1* and *CYP11B2* are different. This work reveals that *HSD3B1*'s different reactivities to AngII and K^+ are likely due to stimulus-selective induction of NGFIB.

Chapter 3: Angiotensin II-regulatable cell-autonomous circadian oscillators in the adrenal zona glomerulosa cells

The mouse *Hsd3b6* shows circadian expression in the adrenal gland, and its expression becomes abnormal when clock genes are deleted (*2*). Thus, this gene is under the control of the internal circadian clock. However, little is known about a molecular clock system within the ZG cells. At least partly because of the multilayered complex structure of the adrenal cortex, functional properties of the local clock in the ZG cells have never been studied. Here I provide evidence that a functional local clock system exists in human and rodent adrenal gland ZG cells. Using live-cell bioluminescence imaging and mRNA expression profiling with laser-captured cells, I illustrate robust circadian expression profiles of canonical clock genes in the ZG cells. Genetic perturbation studies confirm a requirement of clock genes for the autonomous oscillation of the ZG cells. I show that the clocks in the ZG cells exhibit phase-dependent phase shifts in response to AngII stimulation. Thus, AngII stimulation-responsive cell-autonomous circadian oscillators are present in the ZG cells.

References

(*1*) Doi *et al.*, *J Clin Endocrinol Metab* **99**, E257–62, 2014

(*2*) Doi *et al.*, *Nat Med* **16**, 67–74, 2010

(*3*) Ota *et al.*, *Mol Cell Endocrinol* **349**, 30–37, 2012

(*4*) Ota *et al.*, *Mol Cell Biol* **34**, 3880–94, 2014

（続紙 2 ）

（論文審査の結果の要旨）

3β-水酸化ステロイド脱水素酵素（3β-HSD）はアルドステロン合成において必須の酵素である。最近、ヒトにある 2 種の 3β–HSD アイソフォームのうち、HSD3B1 が副腎のアルドステロン産生を担う球状層細胞においては強く発現し、これが、体内時計に制御されることが明らかとなった。申請者は、第一章において、HSD3B1 が NGFIB を介して AngⅡの刺激により誘導されることを見出し、時計のシグナルと *HSD3B1* のプロモーター上で収斂・統合される経路を明らかにした。つづく、第二章において、この NGFIB-*HSD3B1* 経路が K^+には応答しないことを明らかにした。この発見は、AngⅡと K^+が異なる経路でステロイド合成を制御することを分子レベルで実証した初めての研究として注目される。さらに、第三章において、アルドステロンの産生細胞である球状層細胞に機能的な分子時計システムが存在することを証明した。

上述の研究により、レニン-アンジオテンシン-アルドステロン系は、*HSD3B1* のプロモーターに NGFIB を介して直接作用するとともに、アルドステロン細胞内の時計機構を介して間接的にも *HSD3B1* の発現制御に寄与することが分かった。さらに、アルドステロン細胞の *HSD3B1* の発現制御における、AngⅡ、K^+、および体内時計の分子機構を同定した。本論文は、生体時計がレニン-アンジオテンシン-アルドステロン系と形成する多重で選択的な制御機構の一端を初めて明らかにし、近年、注目されるアルドステロンを原因とした高血圧症の解明、治療に、新たな側面を開く画期的な研究と言える。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 27 年 2 月 23 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。

要旨公表可能日： 27 年 5 月 23 日以降