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研究課題名（和文）血圧調節中枢の性差－女性に多い低血圧症の機序解明を目指して－

研究課題名（英文）Sex differences in blood pressure regulation - elucidation of the central mechanisms of hypotension in females -

研究代表者

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研究成果の概要（和文）：自然発症性高血圧ラット（SHR）では安静時血圧値において顕著な性差が認められており、雌は雄に比較し低値である。本研究では血圧中枢（延髄孤束核：NTS）の機能的性差が血圧値の違いに寄与すると考え、NTS機能を支える分子基盤の性差について調べた。その結果、雌のSHRでは雄に比較しTRPV4遺伝子発現が有意に高値であった。TRPV4アゴニスト（4 alpha -PDD）をNTSへ微量注入した結果、血圧の減少が認められた。以上から、SHRではNTSにおけるTRPV4遺伝子発現の性差が血圧水準の性差に寄与している可能性が示唆された。

研究成果の概要（英文）：Female Spontaneous Hypertensive Rats (SHRs) exhibit a lower arterial pressure (AP) than age-matched males but the molecular mechanisms are not well understood. We hypothesized that a different neuronal function between male and female at the level of the nucleus tractus solitarius (NTS), a pivotal region for regulating the set-point of AP, could contribute to the sex difference in cardiovascular homeostasis.Transcriptomic techniques revealed that the transcript of TRPV4 (Transient receptor potential cation channel subfamily V member 4) was significantly up-regulated in the NTS of female SHRs compared to males. Estradiol treatment of ovariectomized females did not affect its expression. The channel was localized in both neurons and glia. TRPV4 agonist 4 alpha-PDD microinjected into the NTS decreased AP and heart rate in both genders. Thus, our results suggest that TRPV4 might be involved in the gender differences of AP in SHRs.

研究分野：　basic biology

キーワード：　blood pressure　SHR　NTS　transcriptomics　sex difference　TRPV4　4alpha-PDD

１．研究開始当初の背景

(1) The level of arterial pressure (AP) is lower in pre-menopausal women than in men of similar age. Although low AP in women is considered an advantage for preventing cardiovascular diseases caused by hypertension, such as, heart attacks, end stage renal failure, and strokes, it also contributes to the negative physiological effects.

(2) Several studies show that sex hormones could play a role, however, the mechanisms responsible for the gender differences in AP control are not fully understood.

(3) Since pre-menopausal women are known to have a lower sympathetic outflow and a greater baroreceptor reflex function, which is a process that is critically important for AP stability, we hypothesized that neuronal functions at the level of cardiovascular centers are different between men and women, and this contributes to the gender differences in cardiovascular homeostasis. Biological sex differences were described in brain structure, function and chemistry, however whether brain cardiovascular centers exhibit gender differences in molecular characteristics and physiological functions has not been fully studied.

(4) The nucleus of the solitary tract (nucleus tractus solitarius, NTS) is the central termination of baroreceptor inputs and a pivotal structure regulating the baroreflex mechanism of blood pressure regulation. Our previous studies using male spontaneously hypertensive rats (SHRs), an animal model of human essential hypertension, and the normotensive Wistar Kyoto (WKY) rat, revealed differences in gene-expression profiles in NTS and demonstrated that the expression of genes coding for inflammatory molecules contributed to the basal levels of AP (Gouraud et al. *Acta Physiol (Oxf)*. 2016, *J Hypertens.* 2011, *Auton Neurosci.* 2011). Thus, we hypothesized that sexual dimorphism of NTS transcripts was one of the fundamental mechanisms underlying the gender-specific regulation of basal AP.

２．研究の目的

In this project we proposed to investigate the central mechanisms underlying gender difference in regulating AP. Our first goal was to identify NTS genes related to gender-dependent variation in AP regulation. Since SHR model exhibits exaggerated gender differences of the basal AP compared to normotensive rats, it was chosen as a model to investigate:

(1) gender differences in NTS gene expression profile.

(2)　the role of estrogen on the expression profile of candidate gender-dependent gene(s) in NTS.

(3) the cellular location of the corresponding protein(s) in the NTS.

(4) the role of the candidate gene(s) on brain functions that control blood pressure AP.

３．研究の方法

(1) Transcriptome screening

Microarray technique was used to screen the transcriptome of NTS and hypothalamus, another cardiovascular center, in SHRs and WKY rats. Lists of differentially expressed (DE) genes between females and males were generated and analyzed to interpret the functional meaning of the various gender-dependent biological processes associated with cellular function in NTS and hypothalamus of both strains. Lists were loaded into EASE (Expression Analysis Systematic Explorer) tool from DAVID (Database for Annotation, Visualization, and Integrated Discovery) Bioinformatics Resources version 6.8 for gene ontology (GO) classification and functional gene annotation clustering.

(2) Validation of gene expression changes

RT-PCR technique and carefully selected reference genes were used to confirm microarray data for the

selected candidate gene.

(3) Estradiol level effect

Female SHRs were ovariectomized and half of them were treated with estradiol for one month by using Alzet minipumps. Total RNA from NTS was extracted and submitted to RT-PCR to assess the expression profiles of the validated candidate gene.

(4) Cell specificity of candidate gene expression

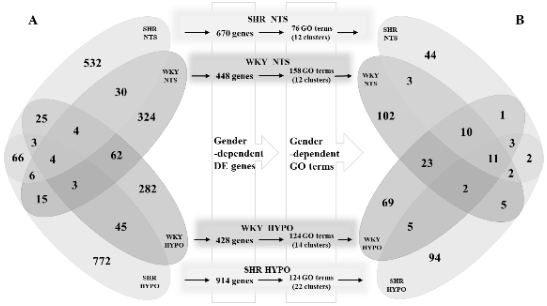
The immunolocalization of candidate protein was determined by using antibodies marker for various cell types including neurons and astrocytes.

(5) Functional examination of the role of candidate molecule in cardiovascular control

Target molecule was injected into the barosensitive area of the NTS of anesthetized SHRs and their blood pressure (AP) and heart rate (HR) were monitored.

４．研究成果

(1) Microarray data analysis

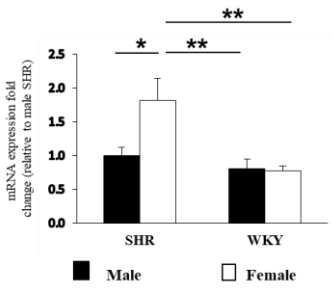


(Fig.1) Shared gender-dependent DE genes and GO terms in NTS and Hypothalamus of SHRs and WKYs (Onishi et al. *Physiol Genomics*, 2018)

Microarray technology was used as an initial screening method to identify groups of DE genes between males and females in SHRs NTS. Hypothalamus of SHRs and WKYs tissues were also used to assess tissue and strain specificity of the variations in gene expression. With a cutoff of Log2 (Fold Change $_{f/m}$) ±1, we identified a total of 532 DE genes in the NTS of SHRs specifically (Fig.1A).

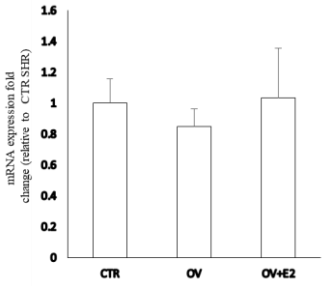
GO categories enrichment study performed on this list with EASE tool revealed a total of 12 clusters of 44 enriched GO terms (Fig.1B) mainly related to regulation of blood pressure, regulation of water and chemical homeostasis, icosanoid and arachidonate transport, and ion transport and that were specifically enriched in SHR NTS. Interestingly, the transcript for TRPV4, a channel known for its involvement in lowering blood pressure, was specifically upregulated in female NTS compared to male NTS in SHRs and was found to be an overlapping gene of 18 different SHR-specific GO terms belonging to seven different clusters.

(2) TRPV4 gene expression



(Fig.2) Gene expression of TRPV4 in the NTS in both genders of SHRs and WKYs (n=7 each, * p<0.05, ** p<0.01) (modified from Onishi et al. *Physiol Genomics*, 2018)

RT-PCR experiments confirmed the upregulation of *TRPV4* transcript in the NTS of female SHRs compared with male SHRs but not in WKYs NTS (Fig.2) nor in the hypothalamus of SHRs and WKYs (data not shown).

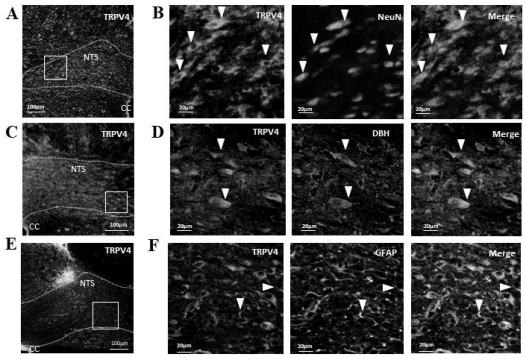


(Fig.3) Effect of ovariectomy (OV) and estradiol treatment (OV+E2) on *TRPV4* gene expression in the NTS of female SHRs (n=6 each)

Our investigation on gene expression differences in ovariectomized female SHRs treated or not with

estradiol for one month showed that estradiol level does not affect *TRPV4* transcript expression level in the NTS (Fig.3).

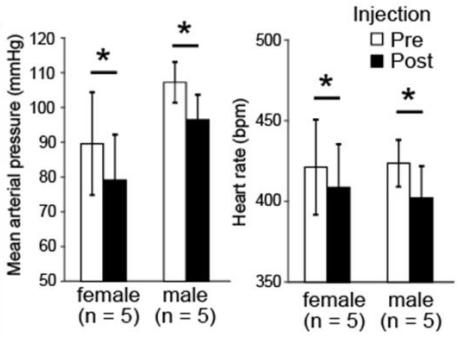
(3) Immunolocalization of TRPV4 in SHR NTS



(Fig.4) Expression of TRPV4 protein in the NTS of SHRs (representative photograph for both genders, modified from Onishi et al. *Physiol Genomics*, 2018)

TRPV4 staining was identified in both male and female SHRs in the barosensitive area of NTS (Fig.4A,C,E) and in surrounding areas, but no apparent differential expression between genders was observed. TRPV4 staining was found mainly in neurons of NTS as shown by its colocalization with NeuN (Fig.4B). TRPV4 was found expressed in a few noradrenergic/adrenergic neurons, as shown by its colocalization with DBH (Fig.4D) in both genders. TRPV4 protein was also identified in astrocytes, as shown by its colocalization with GFAP (Fig.4F) in both genders.

(4) Functional role of TRPV4



(Fig.5) Injection of TRPV4 agonist into the NTS of female and male SHRs induced depressor and bradycardic response (* p<0.05) (from Onishi et al. *Physiol Genomics.* 2018)

We examined the effects of a TRPV4 agonist, 4α-PDD, on AP and HR. 4α-PDD injection into the right NTS of urethane-anesthetized SHRs triggered a significant decrease of both MAP and HR in both male and female SHRs (Fig.5). The decrease of MAP and HR was more important in females than in males, however, the difference was not significant. These findings suggest that, in the NTS, TRPV4 activates projection neurons and induces the depressor and bradycardic responses. We speculated that activation of $TRPV4^{+}$ glutamatergic efferent neurons projecting to and activating CVLM GABAergic neurons results in a blood pressure decrease. Nevertheless, in situ hybridization targeting both glutamatergic marker VGLUT2 and TRPV4 genes is warranted to confirm this hypothesis. In addition, we cannot exclude that glial TRPV4 may also contribute to an alteration of the NTS neuronal activity that regulates AP levels. In fact, activated astrocytes can release gliotransmitters such as glutamate that can enhance neuronal excitability through mGluR activation.

Our findings suggest that an estrogen-independent increased expression of the *Trpv4* transcript in NTS may be associated with low basal AP in female SHRs, although underlying cellular and molecular mechanisms need to be further elucidated. Our findings were presented in various conferences and most of them were recently published in Onishi et al. *Physiol Genomics*, 2018.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　1　件）

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(3) Gouraud S, Onishi M, Yamanaka K, Waki H. Deciphering the transcriptome of hypertensive nucleus tractus solitarii to unmask blood pressure regulation mechanisms. Symposium: Physiological and pathophysiological changes in the transcriptome: New targets for understanding autonomic regulation. International Union of Physiological Sciences (IUPS) 36th world congress, Rio De Janeiro, Brazil. 2017.8.

(4) Onishi M, Takagishi M, Yamanaka K, Miyamoto Y, Waki H, Gouraud S. Gender-specific transcriptomic profiles in rat cardiovascular centers. 94 回日本生理学会大会，浜松, 2017.03.

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Gene Expression Omnibus (GEO) dataset:

(1) Onishi M, Yamanaka K, Miyamoto Y, Waki H, Gouraud SS . Microarray datasets for Gender differences of gene expression profiles in brain cardiovascular centers of SHRs. NCBI's Gene Expression Omnibus (GEO); SuperSeries accession number: GSE107660 (January 2018)

(2) Onishi M, Yamanaka K, Miyamoto Y, Waki H, Gouraud SS . Microarray datasets for Gender differences of gene expression profiles in brain cardiovascular centers of WKY rats. NCBI's Gene Expression Omnibus (GEO); SuperSeries accession number: GSE107689 (January 2018)

６．研究組織

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