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学位(専攻分野)	博士(理学)
学 位 記 番 号	総研大甲第 2050 号
学位授与の日付	平成 30 年9月28日
学位授与の要件	生命科学研究科　生理科学専攻 学位規則第6条第1項該当
学 位 論 文 題 目	Characterization of TRPA1 from disease vector mosquitoes
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(Form 3)

Summary of Doctoral Thesis

Name in full: Li, Tianbang

Title

Characterization of TRPA1 from disease vector mosquitoes

Introduction

Female mosquitoes possess a strong innate drive to find and feed on humans. They efficiently spread blood-borne diseases, causing health crises all over the world. In mosquito host-seeking strategy, heat and odorant sensation are two indispensable factors. And Transient Receptor Potential Ankyrin 1 (TRPA1) is known to be involved in thermotaxis and nociception while it is not related to attraction. When a TRPA1 agonist allyl isothiocyanate (AITC) was administrated to sucrose solution, wild-type *Drosophila melanogaster* showed significant avoidance behaviors compared with its TRPA1-loss-of-function mutants. It was reported that expression of *Anopheles gambiae* (Ag) TRPA1 is restricted to a few heat-sensitive small coeloconic sensilla neurons located in the distal-most region of antenna. In another experiment comparing thermotaxis between wild-type and TRPA1 knock-out *Aedes aegypti* (Aa), it was found that mutant mosquitoes failed to avoid the temperature higher than 43 °C which could dramatically reduce the stay of wild-type mosquitoes. Moreover, TRPA1 variants were found in many species, and AgTRPA1 variants revealed different thermosensitivity. However, no TRPA1 was characterized in other mosquito species and the mechanism for heat-evoked activation of mosquito TRPA1 is not well-understood.

Objectives

I planned to clone and characterize TRPA1 from *Anopheles gambiae*, *Anopheles stephensi* (As), *Aedes aegypti* and *Culex pipiens pallens* (Cp). I want to understand how mosquitoes evolved their sensory systems. I also want to screen specific mosquito TRPA1 agonists for the development of novel mosquito repellents.

Materials and Methods

Total RNA from adult specimen of 4 disease vector mosquito species was extracted as template for reverse transcription to obtain cDNA. Nested PCR primers designed based on the results of 5' and 3' rapid amplification of cDNA ends were used to clone and identify multiple mosquito TRPA1 variants. A Fura-2 calcium-imaging method was employed to roughly examine the responses of TRPA1 in Human Embryonic Kidney-derived 293 (HEK293) cells to extracellular thermal or chemical stimuli. A whole-cell patch-clamp method was used for further confirmation and characterization of TRPA1 channel properties, and current density and temperature threshold for heat activation were measured for evaluating the chemosensitivity and thermosensitivity. Western blotting experiments was performed to quantify the relative expression levels of TRPA1 protein on the cell membrane in which myc-tagged TRPA1 protein was detected with a myc antibody and then it was normalized to the amount of β-actin.

Results

I designed a readily comprehensible nomenclature to describe complicated mosquito TRPA1 variants: according to exon location, I named the upstream exon as "a", and the downstream one was named as "b". Upper case for locating in the N-terminus, lower case for locating in the middle part. Newly cloned and identified mosquito TRPA1 variants are based on such nomenclature, and named as *AgTrpA1Aa, AgTrpA1Ba, AsTrpA1Ab, AsTrpA1Ba, AaTrpA1Ab, AaTrpA1Ba, AaTrpA1Bb, CpTrpA1Ab* and

CpTrpA1Ba. Among these TRPA1 variants, I found that AgTRPA1Ba, AaTRPA1Ba and AaTRPA1Bb have 14 more amino acids located at the N-terminus. These 14 amino acids significantly attenuated the amplitudes of both heat- and chemical-evoked currents. Both calcium-imaging and whole-cell patch-clamp recording data indicated that the ankyrin repeat-transmembrane domain linker region encoded by a single exon is irrelevant to AaTRPA1 thermal responses. In four mosquito species, TRPA1B was found more thermosensitive than TRPA1A. Tropical mosquito species had similar temperature thresholds for heat-evoked activation of around 30 °C, whilst temperate mosquito CpTRPA1B had a remarkably lower threshold. TRPA1B had higher chemosensitivity than TRPA1A in *Anopheles* mosquitoes, while TRPA1A had higher chemosensitivity than in TRPA1B in *Aedes aegypti* and *Culex pipiens pallens*. Moreover, AaTRPA1 and CpTRPA1 also showed higher chemosensitivity compared with *Anopheles* mosquito TRPA1. The relative protein amount of AgTRPA1Aa on cell membrane was higher than that of AgTRPA1Ba. Finally, octanal, nonanal and decanal were found to be novel mosquito TRPA1 agonists.

Discussion

Upon discovering different exons involved in TRPA1 translation, different *TrpA1* transcripts of mosquitoes were cloned. Splicing variants of TRPA1 from disease vector mosquitoes were compared and the very N-terminal domain of mosquito TRPA1 was found to be the structural determinant for modulating channel property. TRPA1 channels of mosquitoes inhabiting in different latitudes of the world revealed different thermosensitivities. And the variants of TRPA1 channels of mosquitoes belonging to different evolutionary clades exhibited distinct sensitivities to electrophiles. Finally, the discovery of new mosquito TRPA1 chemical agonists implied that species-specific mosquito repellents could be developed.

Conclusion

My electrophysiological studies indicated that the N-terminus of mosquito TRPA1 plays an important role in channel property. Thermosensitivity and chemosensitivity comparison among mosquito species implied the adaptation of their TRPA1 to unique geographical environments during evolution.

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Results of the doctoral thesis screening

博士論文審査結果

Name in Full
氏　名　Li, Tianbang

Title
論文題目　Characterization of TRPA1 from disease vector mosquitoes

Transient receptor potential ankyrin 1 (TRPA1)は温度受容や侵害受容を担うカチオン透過型チャネルであり、熱に対する蚊の忌避行動に関わる。本論文は、4種の蚊(*Anopheles gambiae* (Ag), *Anopheles stephensi* (As), *Aedes aegypti* (Aa), *Culex pipens pallens* (Cp))の TRPA1 チャネル遺伝子を同定し、それらの網羅的な電気生理学的特性解析から新たな分子制御機構と蚊の忌避に寄与しうる TRPA1 選択的な小分子化合物（アゴニスト）を見出した興味深い研究である。

Li 氏は、4種の蚊から9つの TRPA1 バリアント遺伝子を単離・同定した。TRPA1 過剰発現 HEK293 細胞株を用いた細胞内 Ca^{2+}イメージングや whole-cell patch clamp 記録の結果、このうち3つの TRPA1B バリアント（AgTRPA1Ba, AaTRPA1Ba, AaTRPA1Bb）において先行研究では見落とされていた 14 残基のアミノ酸が N 末端に存在すること、およびこの 14 アミノ酸が TRPA1 の熱刺激やリガンドであるアリルイソチオシアネート（化学刺激）誘発性のチャネル電流を減弱させることを新たに見出した。更に、①ショウジョウバエの TRPA1 において温度感受性に重要と推定されていたアンキリンリピートと膜貫通ドメイン間のリンカー領域は AaTRPA1 の熱応答に必須でないこと、②TRPA1B の方が TRPA1A より熱感受性が高いこと、③温帯性の蚊（Cp）の TRPA1B チャネルの活性化温度閾値は熱帯性の蚊3種（Ag, As, Aa）の TRPA1B チャネルの閾値より極端に低いこと、④Ag, As 由来の TRPA1B が TRPA1A より化学感受性が強いのに対し Aa, Cp では TRPA1A の方が TRPA1B より化学感受性が強いこと、⑤4 種間で比較した場合に Aa, Cp 由来の TRPA1 のほうが Ag, As 由来 TRPA1 より化学感受性が強いこと、⑥細胞膜における相対的タンパク発現量を比較した結果、AgTRPA1Aa のほうが AgTRPA1Ba より高いこと、などを明らかにした。さらに、蚊の忌避が期待される植物由来の天然候補化合物 23 種類の中から、AgTRPA1 および AaTRPA1 チャネル電流を増大させる3種（octanal, nonanal, decanal）を同定した。

本研究成果は、蚊の TRPA1 チャネル遺伝子を単離・同定し、それらのチャネル特性や N 末端 14 アミノ酸による電流減弱効果を初めて明らかにした知見であるとともに、ヒトにおけるマラリアや疫病感染予防への応用が期待できる蚊 TRPA1 選択的アゴニストの同定まで行った画期的な知見であり、博士学位に相応しい成果と評価できる。