学 位 論 文 要 約

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題　　目　Studies on the Distribution of Cells Expressing Vomeronasal Receptors in the Olfactory Organ of Turtles
（カメ嗅覚器における鋤鼻受容体発現細胞の分布に関する研究）

脊椎動物において，嗅覚受容は嗅覚受容体を介して行われる。嗅覚受容体は 7 回膜貫通型の G タンパク共役型受容体で，匂い受容体，1 型鋤鼻受容体，および 2 型鋤鼻受容体に分類される。哺乳類では，嗅上皮の線毛性嗅細胞が匂い受容体と Gαolf を共発現し，鋤鼻器の微絨毛性嗅細胞が 1 型鋤鼻受容体と Gαi2，2 型鋤鼻受容体と Gαo を共発現する。カメの嗅覚器は上憩室上皮と下憩室上皮からなる。ミシシッピアカミミガメなど多くのカメでは，上憩室上皮に分布する嗅細胞は樹状突起の先端に線毛と微絨毛の両方をもち，下憩室上皮に分布する嗅細胞は樹状突起の先端に微絨毛のみをもつ。嗅細胞の微細形態に基づくと，上憩室上皮に分布する嗅細胞は匂い受容体と鋤鼻受容体の両方を発現し，下憩室上皮に分布する嗅細胞は鋤鼻受容体だけを発現すると考えられる。しかし，カメ嗅覚器の免疫組織化学的解析では，Gαolf と Gαo が上憩室上皮と下憩室上皮の両方に発現し，Gαi1-3 が下憩室上皮に発現することが示されている。このことは，カメの上憩室上皮に分布する嗅細胞が匂い受容体と 2 型鋤鼻受容体の両方を発現し，下憩室上皮に分布する嗅細胞が鋤鼻受容体に加えて匂い受容体も発現することを示唆する。下憩室上皮における嗅覚受容体の発現について，嗅細胞の微細形態に基づく推測と G タンパク発現との矛盾を解消するには，嗅覚受容体そのものの発現を明らかにする必要がある。そこで本研究では，RT-PCR と *in situ* hybridization によって，カメの嗅覚器における鋤鼻受容体遺伝子の発現を解析した。

カメにおいて，鋤鼻受容体は 2 つの 2 型鋤鼻受容体遺伝子（V2R1 と V2R26）および 2 つの 1 型鋤鼻受容体遺伝子（V1R3 と V1RA14）にコードされている。第 2 章では，2 型鋤鼻受容体遺伝子発現細胞の分布を調べた。RT-PCR 解析ではミシシッピアカミミガメ嗅覚器における 2 型鋤鼻受容体遺伝子の発現が示された。さらに，*in situ* hybridization 解析では 2 型鋤鼻受容体遺伝子が少数の細胞に発現することが示された。V2R1 遺伝子発現細胞は下憩室上皮にのみ存在したが，V2R26 遺伝子発現細胞は上憩室上皮と下憩室上皮の両方に存在した。V2R26 発現細胞の密度は下憩室上皮のほうが上憩室上皮よりも著しく高かった。次に，スッポンの嗅覚器における 2 型鋤鼻受容体遺伝子の発現を調べた。スッポンの嗅覚器には微絨毛性嗅細胞が見つからないにも関わらず，RT-PCR 解析では下憩室上皮における V2R1 遺伝子の発現や上憩室上皮と下憩室上皮における V2R26 遺伝子の発現が明らかになった。一方，*in situ* hybridization 解析では少数の V2R26 発現細胞が主に下憩室上皮で見つかり，V2R1 発現細胞は検出されなかった。第 2 章の結果は，カメの嗅覚器では主に下憩室上皮に分布する少数の嗅細胞が 2 型鋤鼻受容体遺伝子を発現することを示している。

第 3 章では，1 型鋤鼻受容体遺伝子発現細胞の分布を調べた。RT-PCR 解析はアカミミガメの上憩室上皮と下憩室上皮に V1R3 遺伝子が発現することを示し，V1RA14 遺伝子は検

出されなかった。一方，スッポンは V1R3 遺伝子を欠き，V1RA14 遺伝子の発現は嗅覚器で検出されなかった。*In situ* hybridization 解析はアカミミガメの上憩室上皮と下憩室上皮において少数の嗅細胞に V1R3 が発現することを示した。上憩室上皮と下憩室上皮における V1R3 発現細胞の割合は個体によって差があった。

嗅細胞の微細形態によれば，アカミミガメの下憩室上皮において多くの嗅細胞は鋤鼻受容体遺伝子を発現すると考えられるが，本研究の第 2 章と第 3 章で示された少数の嗅細胞における鋤鼻受容体遺伝子の発現は，未知の鋤鼻受容体遺伝子の存在と下憩室上皮に分布する微絨毛性嗅細胞にそれらの遺伝子が発現している可能性を示唆する。そこで，第 4 章ではアカミミガメ嗅覚器において鋤鼻受容体のシグナル伝達に関与する TRPC2 遺伝子の発現を調べた。TRPC2 発現細胞の密度は上憩室上皮と下憩室上皮における鋤鼻受容体発現細胞の密度とほぼ同じであった。従って，鋤鼻受容体は少数の嗅細胞に発現し，カメ嗅覚器に発現する他の鋤鼻受容体遺伝子はおそらく存在しないことが示唆された。さらに，カメ嗅覚器で多くの嗅細胞に発現する嗅覚受容体の種類を確認するために，匂い受容体のシグナル伝達に関与する CNGA2 遺伝子の発現を調べた。*In situ* hybridization 解析ではアカミミガメの上憩室上皮と下憩室上皮の広い範囲に CNGA2 遺伝子の発現が示され，カメ嗅覚器において多くの嗅細胞は匂い受容体遺伝子を発現していることが示唆された。

以上のように，カメ嗅覚器における嗅覚受容体の発現を明らかにすることを目的とした本研究によって，鋤鼻受容体遺伝子は限られた数の嗅細胞に発現することが示された。また，多くの嗅細胞には匂い受容体が発現し，カメにおける嗅覚受容には鋤鼻受容体よりも匂い受容体が主に関与していることが示唆された。さらに，嗅細胞の微細形態と嗅覚受容体の遺伝子発現との関係は，カメと他の脊椎動物との間では保存されていないことが明らかになった。

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In vertebrates, olfactory chemoreception is mediated by olfactory receptors. They are seven transmembrane, G protein coupled receptors including odorant receptors (ORs) coupled to Gαolf, type 1 and type 2 vomeronasal receptors (V1Rs and V2Rs) coupled to Gαi2 and Gαo, respectively. In mammals, the ORs are expressed by ciliated olfactory receptor neurons (ORNs) in the olfactory epithelium, while the V1Rs and V2Rs are expressed by microvillous ORNs in the vomeronasal organ. In turtles, the olfactory organ consists of upper (UCE) and lower chamber epithelia (LCE), lining the upper and lower portion of the nasal cavity, respectively. In many turtles including red-eared sliders, ORNs in the UCE bear both cilia and microvilli on their dendritic knob, while those in the LCE bear only microvilli. Based on the fine structure of ORNs, it can be speculated that the ORNs in the UCE express both ORs and vomeronasal receptors (VRs), while those in the LCE express only VRs. Nevertheless, immunohistochemical analyses of turtle olfactory organ have demonstrated the expression of Gαolf and Gαo both in the UCE and LCE. Moreover, expression of Gαi1-3 has been shown in the LCE. These findings suggest that ORNs in the UCE of turtles express both ORs and V2Rs, whereas those in the LCE express ORs in addition to VRs. The inconsistency between the olfactory receptors deduced by the fine structure of ORNs and those by the G protein expression in the LCE of turtles lead to the need for the elucidation of olfactory receptor genes expressed in the olfactory organ of turtles. Thus, the present study examined the expression of genes encoding VRs using reverse transcriptase-polymerase chain reaction (RT-PCR) and *in situ* hybridization.

In turtles the VRs were encoded by two V2R genes (V2R1, V2R26) and two V1R genes (V1R3, V1RA14). In chapter 2, the distribution of cells expressing V2Rs was investigated. Initially, I sought to determine if the V2R genes are expressed in the olfactory organ of red-eared slider. Total RNA was extracted from the olfactory organ and the first-strand cDNA was synthetized by reverse transcription. To amplify V2R sequences from red-eared slider olfactory organ, primers were designed based on the nucleotide sequences of two V2R genes in western painted turtle. RT-PCR analysis confirmed the expression of V2R genes in the olfactory organ of red-eared slider. Moreover, *in situ* hybridization analysis was performed to clarify the localization of cells expressing V2Rs. Digoxigenin-labelled sense and antisense cRNA probes for gene encoding V2Rs were hybridized with frozen sections of olfactory organ. Cells expressing V2R1 were present only in the LCE, whereas those expressing V2R26 were present both in the UCE and LCE. The density of cells expressing V2R26 was significantly higher in the LCE than that in the UCE. Moreover, V2R genes were expressed by a small number of cells and a region with higher density of cells expressing V2Rs was not found along the

rostro-caudal axis of olfactory organ. Subsequently, the expression of gene encoding V2Rs were investigated in the olfactory organ of soft-shelled turtle. Primers were designed based on the nucleotide sequence of V2R genes in soft-shelled turtle. Despite the fact that microvillous ORNs are not found in the olfactory organ of soft-shelled turtle, RT-PCR analysis revealed the expression of V2R1 genes in the LCE, whereas V2R26 was expressed both in the UCE and LCE. On the other hand, *in situ* hybridization analysis indicated the sparse expression of V2R26 both in the UCE and LCE, while cells expressing V2R1 were not detected. As in the case of red-eared slider, the density of cells expressing V2R26 gene was significantly higher in the LCE than that in the UCE. The results in chapter 2 indicated the expression of V2R genes by a small number of ORNs mainly in the LCE of turtles.

In chapter 3, the localization of cells expressing V1R genes was investigated. RT-PCR analysis indicated the expression of V1R3 gene both in the UCE and LCE of red-eared slider but not that of the V1RA14. On the other hand, V1R3 is lacking in soft-shelled turtle, and the expression of V1RA14 gene was not detected in the olfactory organ. *In situ* hybridization analysis indicated the expression of V1R3 gene by a small number of cells both in the UCE and LCE of red-eared sider. The relative abundance of cells expressing V1R3 gene between the UCE and LCE varied among individuals. Moreover, cells expressing V1R3 gene were almost evenly distributed along the rostro-caudal axis of olfactory organ and a region with higher density of cells expressing V1R3 was not found.

Although the fine structure of ORNs implies the expression of VR genes by a higher number of ORNs in the LCE of red-eared slider, results in the chapter 2 and 3 of the present study indicated the expression of VR genes by a small number of ORNs, implying the presence of unknown genes encoding VRs and their expression by microvillous ORNs in the LCE. Thus, I further examined the expression of genes encoding transient receptor potential cation channel, subfamily c member 2 (TRPC2), an ion channel mediating signal transduction for VRs, in the olfactory organ of red-eared slider in chapter 4. As in the case of VR genes, TRPC2 gene was expressed by a small number of cells. The density of cells expressing TRCP2 was almost equal to that of the cells expressing VRs both in the UCE and LCE. A region with higher density of cells expressing TRPC2 was not found in the olfactory organ of red-eared slider. Moreover, the distribution of cells expressing TRPC2 were closely resembled that of VRs. The cells expressing TRPC2 and VRs were present mainly middle to basal half of epithelium where the nuclei of ORNs exist. These findings suggest the expression of VR genes by a small number of ORNs and that no additional VR genes are likely to present in the olfactory organ of turtle. Furthermore, expression of the gene encoding cyclic nucleotide gated ion channel alpha 2 (CNGA2), an ion channel mediating signal transduction for ORs, was investigated to proxy the type of olfactory receptors expressed by the majority of ORNs in the olfactory organ of turtles. *In situ* hybridization analysis indicated the extensive expression of gene encoding CNGA2 both in the UCE and LCE of red-eared slider, implying the expression of OR genes by the majority of ORNs in the olfactory organ of turtles.

In conclusion, by the present study aimed to determine the type of olfactory receptors expressed in the olfactory organ of turtles it has been shown that the VR genes are expressed by a limited number of ORNs, while the majority of ORNs have been suggested to express OR genes. Thus, it is almost

certain that olfactory chemoreception in turtles is mediated mainly by the ORs rather than the VRs. Moreover, it appears that the correlation between the fine structure of ORNs and the gene expression of olfactory receptors is not conserved among turtles and other vertebrates.