（様式第３号）

学 位 論 文 要 旨

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題目: Cloning and molecular pharmacological characterization of a β-adrenergic-like octopamine receptor from the silkworm *Bombyx mori*
（カイコ *Bombyx mori* のβ－アドレナリン様オクトパミン受容体のクローニング及び分子薬理学的特性）

Octopamine (OA) is a physiologically important invertebrate biogenic amine that has structural and functional similarities to the vertebrate biogenic amines adrenaline and noradrenaline. Following its initial discovery in octopus posterior salivary glands, OA was found in high concentrations in both neuronal and non-neuronal tissues of most invertebrate species studied. OA is synthesized by β-hydroxylation of tyramine (TA), which is a phenolamine synthesized from the amino acid tyrosine by decarboxylation. OA is engaged in sensory inputs, rhythmic behaviors, endocrine regulation, mobilization of lipids and carbohydrates, sleep and aggression as well as more complex physiological events, such as learning and memory, as a neurotransmitter, neuromodulator or neurohormone.

The first insect OA receptor was isolated from the fruit fly *Drosophila melanogaster*. The OA receptor falls into the largest family (rhodopsin receptor family or family A) in the superfamily of G protein-coupled receptors (GPCRs), which share a common structural hallmark containing seven- transmembrane domains (TMs) and associated extracellular and intracellular loops (ICLs). The actions of OA are mediated by interactions with seven-transmembrane G protein-coupled receptors (GPCRs), which induce the production or release of intracellular second messengers such as cAMP and Ca^{2+} via the activation of discrete G proteins and linked effectors. Following the initial isolation in fruit flies, a variety of OA receptors were cloned from several other insect species. To classify the various OA receptors, Evans and Maqueira (2005) proposed a novel classification system in which OA receptors are designated as α-adrenergic-like OA receptors (OctαRs), β-adrenergic-like OA receptors (OctβRs) or OA/TA (or tyraminergic) receptors. This classification is based on the similarities of these proteins to vertebrate adrenergic receptors in terms of amino acid sequence and signaling pathway. Activation of α-adrenergic-like OA receptors expressed in cell lines primarily leads to an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), whereas activation of β-adrenergic-like OA receptors induces an increase in intracellular cAMP concentration ($[cAMP]_i$) but no increase in $[Ca^{2+}]_i$. Activation of the third-class *Drosophila* phenolamine receptor was shown to lead to both a reduction of $[cAMP]_i$ and generation of a Ca^{2+} signal. As OA and TA were equipotent in generating Ca^{2+} signaling, the third-class receptor was designated as the OA/TA receptor. However, members of the third receptor class, which have been identified to date from various insect species, were preferentially activated by TA to reduce $[cAMP]_i$, so the OA/TA class of receptors is now thought to represent a class of TA receptors.

Most of the OA receptors isolated so far belong to the class of α-adrenergic-like OA receptors. We have reported the cloning of an α-adrenergic-like OA receptor (BmOAR1) from the silkworm *Bombyx mori*. Agonist stimulation of BmOAR1 increased both $[Ca^{2+}]_i$ and $[cAMP]_i$ probably by activating the coupled G_q and G_s proteins, respectively. To date, however, only β-adrenergic-like OA receptors from *D. melanogaster* have been cloned. In the present study, we cloned a gene coding for a β-adrenergic-like OA receptor (hereafter designated as BmOAR2) from *B. mori* and

generated a HEK-293 cell line that stably expressed BmOAR2 to compare the functional and pharmacological properties of the second β-adrenergic-like insect OA receptor with its *Drosophila* counterpart.

A cDNA encoding a seven-transmembrane receptor (BmOAR2) was cloned from the nerve tissues of silkworm (*Bombyx mori*) larvae. Sequence analysis indicated that the gene is an ortholog of CG6989, which encodes a *Drosophila* β-adrenergic-like octopamine (OA) receptor (DmOctβ2R). As very little information is available regarding this class of receptors, I generated a cell line that stably expressed the gene in HEK-293 cells to perform functional and pharmacological studies of this receptor. [^{3}H]OA-binding assays using membrane preparations of this cell line showed that the receptor possesses a higher affinity for OA than for tyramine (TA) or dopamine (DA). The cell line elicited a bell-shaped, OA concentration-dependent increase in intracellular cAMP levels, with a maximum at 100 nM. (*R*)-OA was more potent than (*S*)-OA. TA and DA had weak or marginal effects on cAMP production. The OA receptor agonist demethylchlordimeform elicited a similar biphasic response, and the maximum response was attained at a concentration as low as 1 nM. The rank order of potency of other agonists was as follows: naphazoline > tolazoline, clonidine, which is consistent with that of the classical type-2A OA receptors but not type-1 OA receptor, in which clonidine is the most potent agonist. Of the tested antagonists, only chlorpromazine significantly attenuated the OA-induced increase in cAMP levels, which showed that BmOAR2 pharmacologically resembles the classical type-1 OA receptors but not type-2 OA receptors, for which chlorpromazine is a weak antagonist. However, phentolamine, which is one of the most potent type-1 OA receptor antagonists, had no antagonist effect on BmOAR2 but instead had weak agonist activity. No increase in intracellular Ca^{2+} levels was observed with OA at concentrations up to 100 μM.

To identify the amino acid residues interacting with the intrinsic agonist (*R*)-OA in BmOAR2, the wild-type receptor and seven mutant receptors with an amino acid substitution at a potential orthosteric site were expressed in HEK-293 cells and examined for their abilities to elevate intracellular cAMP levels ($[cAMP]_i$) in response to (*R*)-OA. The S206A mutant receptor retained the ability to increase $[cAMP]_i$ after (*R*)-OA treatment, although the stimulated cAMP level was lower than that of the wild-type receptor. In contrast, the other six mutant receptors (D115A, S202A, Y300F, Y300N, Y300L, and Y300A) lacked the ability to elevate $[cAMP]_i$. These results indicate that D115, S202, and Y300 participate in (*R*)-OA binding and the activation of BmOAR2. Homology modeling and docking simulation studies suggest that S202 and Y300 interact with the phenolic OH group of (*R*)-OA, whereas D155 interacts with the β-OH group and the NH_2 group of (*R*)-OA.

I employed the active metabolite DMCDM of the classical formamidine insecticide chlordimeform, which is an OA receptor agonist and leads to hyperactivity and death in insects, to clarify its pharmacological effect on HEK293-BmOAR2 cell lines. It was suggested that the change in $[cAMP]_i$ played a significant role in the insecticidal effect of DMCDM, rather than the change in $[Ca^{2+}]_i$, because DMCDM approximately 400-fold more selectively coupled to G_s protein-adenylate cyclase pathway to elevate intracellular cAMP levels, than G_q protein-phospholipase C-inositol triphosphate (IP_3) pathway to generate increased levels of intracellular Ca^{2+} in BmOAR1. I found in this study that the maximal cAMP level induced by DMCDM was attained at a concentration 1 nM, and the EC_{50} value of DMCDM was estimated to be approximately 0.1 nM in HEK293-BmOAR2 cell line, while in the assay of DMCDM using HEK293-BmOAR1 cell line, the EC_{50} (mean ± S.E.) was 234 ± 48 nM and the maximal cAMP level in DMCDM was attained at a concentration 10 μM. Since DMCDM is much more potent in BmOAR2 than in BmOAR1, I suggest that the main target of chlordimerform might be β-adrenergic-like OARs. The cloned receptor described in this study is a β-adrenergic-like OA receptor with unique functional and pharmacological properties, and these studies on the orthosteric site of BmOAR2 should provide more opportunities to discover novel insect control chemicals.