Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres


Research Report

# Expression of ocular albinism 1 (OA1), 3, 4- dihydroxy- L-phenylalanine (DOPA) receptor, in both neuronal and non-neuronal organs

*Nobuhiko Fukuda*[a,1], *Saki Naito*[a,1], *Daiki Masukawa*[a], *Moemi Kaneda*[a], *Hiroshi Miyamoto*[a,b], *Takaya Abe*[c], *Yui Yamashita*[c], *Itaru Endo*[b], *Fumio Nakamura*[a,*], *Yoshio Goshima*[a,*]

[a]*Department of Molecular Pharmacology and Neurobiology, Yokohama City University Graduate School of Medicine, Yokohama 236-0004, Japan*

[b]*Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Yokohama 236-0004, Japan*

[c]*Laboratory for Animal Resources and Genetic Engineering, RIKEN Center for Developmental Biology, Kobe 650-0047, Japan*

ARTICLE INFO

*Article history:*
Accepted 10 January 2015
Available online 16 January 2015

*Keywords:*
DOPA
OA1
GPR143
Knockout mice
Immunohistochemistry

ABSTRACT

*Oa1* is the casual gene for ocular albinism-1 in humans. The gene product OA1, alternatively designated as GPR143, belongs to G-protein coupled receptors. It has been reported that OA1 is a specific receptor for 3, 4-dihydroxy- L-phenylalanine (DOPA) in retinal pigmental epithelium where DOPA facilitates the pigmentation via OA1 stimulation. We have recently shown that OA1 mediates DOPA-induced depressor response in rat nucleus tractus solitarii. However, the distribution and function of OA1 in other regions are largely unknown. We have generated *oa1* knockout mice and examined OA1 expression in both neuronal and non-neuronal tissues by immunohistochemical analyses using anti-mouse OA1 monoclonal antibodies. In the telencephalon, OA1 was expressed in cerebral cortex and hippocampus. Predominant expression of OA1 was observed in the pyramidal neurons in these regions. OA1 was also expressed in habenular nucleus, hypothalamus, substantia nigra, and medulla oblongata. The expression of OA1 in the nucleus tractus solitarii of medulla oblongata may support the reduction of blood pressure by the microinjection of DOPA into this region. Outside of the nervous system, OA1 was expressed in heart, lung, liver, kidney and spleen. Abundant expression was observed in the renal tubules and the splenic capsules. These peripheral regions are innervated by numerous sympathetic nerve endings. In addition, substantia nigra contains a large population of dopaminergic neurons. Thus, the immunohistochemical analyses suggest that OA1 may modulate the monoaminergic functions in both peripheral and central nervous systems.



*Corresponding authors. fax: +81 45 785 3645.
E-mail addresses: f-nakamura@umin.ac.jp (F. Nakamura), goshima@med.yokohama-cu.ac.jp (Y. Goshima).
[1]These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.brainres.2015.01.020
0006-8993/

## 1. Introduction

It has been widely accepted that 3,4- dihydroxy- L-phenylalanine (DOPA) is a precursor for catecholamines and DOPA is immediately converted to dopamine by the enzyme aromatic L-amino acid decarboxylase (AADC) in both neuronal and non-neuronal organs (Nagatsu, 1991). However, recent accumulated evidence suggests that DOPA may act as a neurotransmitter in the central nervous system (Misu and Goshima, 1993; Misu et al., 1995; Mons et al., 1988). The evidence almost fulfills the classical criteria of neurotransmitters including biosynthesis, presence, metabolism, active transport, physiological release, competitive antagonism, physiological and/or pharmacological responses and specific receptors. DOPA is generated from tyrosine with tyrosine hydroxylase (TH) (Nagatsu, 1995). Immunohistochemical studies show the existence of TH-positive but AADC-negative neurons in the nucleus tractus solitarii (NTS) and dorsal motor vagal nucleus complex area (Karasawa et al., 1991; Meister et al., 1988). This suggests that these neurons may contain DOPA as an end product, per se, a neurotransmitter. Electrical field stimulation on rat striatal slices evoked the release of DOPA (Goshima et al., 1988, 1993, 1996), which was tetrodotoxin-sensitive and $Ca^{2+}$ dependent. This suggests that DOPA may be released in a transmitter-like manner. In addition, DOPA microinjection into NTS reduced blood pressure and heart rate (Kubo et al., 1992). This response was competitively antagonized by the pretreatment with the microinjection of DOPA ester compounds, DOPA-methylester or DOPA-cyclohexylester (DOPA-CHE), into NTS (Furukawa et al., 2000). Thus, DOPA may act as a neurotransmitter in the central nervous system.

Recently, Lopez et al. reported that OA1 (ocular albinism-1), alternatively designated as GPR143, is a specific receptor for DOPA in the retinal pigmental epithelium (Lopez et al., 2008). OA1 has been studied as the gene product of *oa1*, which is the causal gene for ocular albinism 1, an x-linked disorder of retinal hypopigmentation (Bassi et al., 1995). OA1 protein is localized to the melanosomes in retinal pigmental epithelium and controls their maturation (Giordano et al., 2009; Schiaffino and Tacchetti, 2005). The *oa1* mutation leads to giant melanosomes in retinal pigmental epithelium in turn to bring the ocular albinism. OA1 belongs to G-protein-coupled receptor (GPCR) family (Sone and Orlow, 2007). DOPA stimulation on OA1 increases the intracellular calcium concentration via Gq-activation (Lopez et al., 2008). In addition, we have recently reported that mouse OA1 binds DOPA and this binding is competitively antagonized by DOPA-CHE (Hiroshima et al., 2014). The knockdown of *OA1* in rat NTS blunted the DOPA-induced blood pressure reduction. In addition, we have also shown OA1 expression in the wide-range of CNS regions, including olfactory bulb, cerebral cortex, hippocampus, hypothalamus, and substantia nigra (Masukawa et al., 2014). These data suggest that besides the retinal pigmental epithelium, neuronal and/or non-neuronal cells may utilize OA1 as DOPA receptors.

To explore the expression and function of OA1 in vivo, we generated *oa1* knockout mice and investigated the expression of OA1 in mouse various organs with anti-OA1 monoclonal antibody (mAb). OA1 was expressed in both neuronal and non-neuronal tissues. Given that knockdown of OA1 mRNA in rat NTS suppressed the DOPA-induced response (Hiroshima et al., 2014), OA1 may be involved in not only retinal pigmentation but alternate functions in both neuronal and non-neuronal tissues.

## 2. Results

### 2.1. *Generation of Oa1 knockout mice*

*Oa1 (gpr143)* knockout mice were generated with two-step strategy. Firstly, floxed-gpr143 mice were generated. In these mice, one loxP motif and a neomycin-resistance gene were inserted into the 5′ side of exon 1 of mouse *oa1 (gpr143)* gene and the other loxP motif was inserted into the 3′ side of the exon 1 (Fig. 1A). Genomic Southern blotting and genotype PCR confirmed the homologous recombination in the floxed-gpr143 mice (Fig. 1B, C). Secondly, the floxed-gpr143 mice were intercrossed with the CAG-Cre mice (Sakai and Miyazaki, 1997) to generate *oa1* knockout mice, in which the exon 1 of *oa1* was deleted (Fig. 1A). The deletion was confirmed by genotype PCR (Fig. 1C). Since mouse *oa1* gene is localized in X chromosome, hemizygous male mice (*oa1 -/y*) and homozygous female mice (*oa1 -/-*) are *oa1* knockout mice. Both *oa1-/y* and *oa1 -/-* were viable. We did not detect any obvious gloss defect in these knockout mice. However, using ophthalmoscopy, we found irregular hypopigmentation in the retinae of *oa1-/y* mice comparing to those of wild-type mice (Fig. 2A and B). This represents that the disruption of *oa1* gene brings the hypopigmentation of retinal epithelium, confirming the earlier report (Incerti et al., 2000).

### 2.2. *OA1 mRNA expression in mouse various tissues*

To survey the OA1 expression patterns in various organs, we examined the mRNA expression of *OA1* in adult mouse tissues by RT-PCR (Fig. 3). In the central nervous system, olfactory bulb, cerebral cortex, corpus striatum, hypothalamus, hippocampus, mid-brain, cerebellum and lower brain stem were expressed *OA1* mRNA. The strong signal was observed in cerebral cortex and hypothalamus. Olfactory bulb, hippocampus, midbrain and lower brain stem showed moderate expression. On the other hand, the limited expression of *OA1* mRNA was observed in corpus striatum and cerebellum. In non-neuronal organs, *OA1* mRNA was expressed in heart, liver, kidney, lung and spleen (Fig. 3). Notably, the expression in kidney was more abundant than any other organs. The expression level in lung was comparable to those in cerebral cortex and hypothalamus. Thus, the broad expression of *OA1* suggests that this receptor may participate in the various physiological functions other than the retinal pigmentation.

### 2.3. *OA1 expression in cerebral cortex and hippocampus*

We next examined immunohistochemical analysis of OA1 in mouse brain with anti-OA1 rat mAb, which was raised against the carboxyl terminal region (314-405 AA) of mouse OA1. To demonstrate the specific immunoreactive signals, sections from both wild-type and *oa1-/y* mice were stained with the anti-OA1 mAb in the exactly same conditions. The immunoreactive signal was observed in the cerebral cortex of wild-type mouse